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A TEXT-BOOK
OF
PHYSIOLOGICAL CHEMISTRY.

BY
OLOF HAMMARSTEN,
Late Professor of Medical and Physiological Chemistry in the University of Upsala.

Authorized Translation

FROM THE AUTHOR'S ENLARGED AND REVISED SIXTH GERMAN EDITION

BY
JOHN A. MANDEL, Sc.D.,
Professor of Chemistry in the New York University and Bellevue Hospital Medical College.

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TRANSLATOR'S PREFACE TO THE FIFTH AMERICAN EDITION.

SINCE the appearance of the First American Edition of Hammarsten's Physiologischen Chemie in 1893 it has become more and more popular as a text-book in our medical schools, and has acquired much favor among the biochemical research workers of this country.

A special debt of gratitude is owing to Professor Hammarsten for his many revisions, which have enabled this work to keep pace with all the advances of this department of chemical science. The same sound critical survey of the subject characterizes this as well as the past editions.

I am under obligations to Dr. Charles B. Robinson for much assistance in proof revision.

JOHN A. MANDEL.

NEW YORK, December, 1907.

59374

PREFACE TO THE SIXTH GERMAN EDITION.

ALTHOUGH only a short time has elapsed since the appearance of the Fifth Edition, the enormous advances in the different domains of physiological chemistry have necessitated a thorough revision of each of the chapters and a rewriting of most of them. Because of these facts it has been impossible to prevent an increase in the size of the book, and hence this edition is somewhat larger than the preceding ones. Acceding to the wishes expressed by many friends, an Authors Index has been added to this edition. but in other respects the plan of the work has not been changed.

OLOF HAMMARSTEN.

UPSALA, September 8, 1906.

CONTENTS.

CHAPTER I.

PAGE
INTRODUCTION 1

CHAPTER II.

THE PROTEIN SUBSTANCES 26

CHAPTER III.

THE CARBOHYDRATES 104

CHAPTER IV.

THE ANIMAL FATS 131

CHAPTER V.

THE ANIMAL CELL 139

CHAPTER VI.

THE BLOOD 170

CHAPTER VII.

CHYLE, LYMPH, TRANSUDATES, AND EXUDATES 250

CHAPTER VIII.

THE LIVER 280

CHAPTER IX.

DIGESTION 339

CHAPTER X.

TISSUES OF THE CONNECTIVE SUBSTANCE 428

CHAPTER XI.

PAGE
MUSCLES.. 447

CHAPTER XII.

BRAIN AND NERVES.. 479

CHAPTER XIII.

ORGANS OF GENERATION.. 495

CHAPTER XIV.

THE MILK.. 514

CHAPTER XV.

THE URINE.. 541

CHAPTER XVI.

THE SKIN AND ITS SECRETIONS.. 685

CHAPTER XVII.

CHEMISTRY OF RESPIRATION.. 696

CHAPTER XVIII.

METABOLISM.. 715

GENERAL INDEX.. 779

INDEX TO AUTHORS.. 813

PHYSIOLOGICAL CHEMISTRY.

CHAPTER I.

INTRODUCTION.

It follows from the law of the conservation of matter and of energy that living beings, plants and animals, can produce neither new matter nor new energy. They are only called upon to appropriate and assimilate already existing material and to transform it into new forms of energy.

Out of a few relatively simple combinations, especially carbon dioxide and water, together with ammonium compounds or nitrates, and a few mineral substances, which serve as its food, the plant builds up the extremely complicated constituents of its organism, proteins, carbohydrates, fats, resins, organic acids, etc. The chemical work which is performed in the plant must therefore, in the majority of cases, consist in syntheses; but besides these, processes of reduction take place to a great extent. The radiant energy of the sunlight induces the green parts of the plant to split off oxygen from the carbon dioxide and water, and this reduction is generally considered as the starting-point of the following syntheses. According to a hypothesis suggested by A. Baeyer,[1] at first formaldehyde is produced, $CO_2+H_2O=CH_2O+O_2$, which by condensation is transformed into sugar, and this then serves in the structure of other bodies. The energy of the sun, which produces this splitting, is not lost; it is only transformed and is stored as chemical energy in the new compounds produced in the synthesis. W. Loeb[2] has been able to obtain formaldehyde as a direct reaction product from CO_2 and H_2O by the aid of the silent electric discharge. The formation of aldehyde takes place in the three following phases: first, $2CO_2=2CO+O_2$; second, $CO+H_2O=CO_2+H_2$; and third, $CO+H_2=HCOH$. The formation of sugar from CO_2 and H_2O with the introduction of energy can be expressed by the following:

---

[1] Ber. d. d. chem. Gesellsch., 3.

[2] Zeitschr. f. Elektrochem., 12.

1. $CO_2+H_2O=CO+H_2+O_2$.
2. $H_2+CO=HCOH$.
3. $2(H_2+CO)=CH_2OH.CHO$.
4. $6HCOH=C_6H_{12}O_6$.
5. $3CH_2OH.CHO=C_6H_{12}O_6$.

In animal life the conditions are not the same. Animals are dependent either directly, as the herbivora, or indirectly, as the carnivora, upon plant-life, from which they derive the three chief groups of organic nutritive matter—proteins, carbohydrates, and fats. These bodies, of which the protein substances and fats form the chief mass of the animal body, undergo within the animal organism a cleavage and oxidation, and yield as final products exactly the above-mentioned chief components in the nutrition of plants, namely, carbon dioxide, water, and ammonia derivatives, which are rich in oxygen and have little energy. The chemical energy, which is partly represented by the free oxygen and partly stored up in the above-mentioned more complex chemical compounds, is transformed into other forms of energy, principally heat and mechanical work. While in the plant we find chiefly reduction processes and syntheses, which by the introduction of energy from without produce complex compounds having a greater content of energy, we find in the animal body the reverse of this, namely, cleavage and oxidation processes, which, as we used to state, convert chemical tension into living force.

This difference between animals and plants must not be overrated, nor must we consider that there exists a sharp boundary-line between the two. This is not the case. There are not only lower plants, free from chlorophyll, which in regard to chemical processes represent intermediate steps between higher plants and animals, but the difference existing between the higher plants and animals is more of a quantitative than of a qualitative kind. Plants require oxygen as peremptorily as do animals. Like the animal, the plant also, in the dark and by means of those parts which are free from chlorophyll, takes up oxygen and eliminates carbon dioxide, while in the light the oxidation processes going on in the green parts are overshadowed or hidden beneath the more intense reduction processes. As in the animal, we also find a heat production in fermentation produced by plant organisms; and even in a few of the higher plants—as the *aroideæ* when bearing fruit—a considerable development of heat has been observed. On the other hand, in the animal organism, besides oxidation and splitting, reduction processes and syntheses also takes place. The contrast which seemingly exists between animals and plants consists merely in that in the animal organism the processes of oxidation and splitting are predominant, while in the plant chiefly those of reduction and synthesis have thus far been studied.

WÖHLER[1] in 1824 was the first to observe an example of SYNTHETICAL PROCESSES within the animal organism. He showed that when benzoic acid is introduced into the stomach it reappears as hippuric acid in the urine, after combining with glycocoll (aminoacetic acid). Since the discovery of this synthesis, which may be expressed by the following equation:

$$\underset{\text{Benzoic acid}}{C_6H_5.COOH} + \underset{\text{Glycocoll}}{NH_2.CH_2.COOH} = \underset{\text{Hippuric acid}}{NH(C_6H_5.CO).CH_2.COOH} + H_2O,$$

and which is ordinarily considered as a type of an entire series of syntheses occurring in the body where water is eliminated, the number of known syntheses in the animal kingdom has increased considerably. Many of these syntheses have also been artificially produced outside of the organism, and numerous examples of animal syntheses of which the course is absolutely clear will be found in the following pages. Besides these well-studied syntheses, there occur in the animal body also similar processes unquestionably of the greatest importance to animal life, but of which we know nothing with positiveness. We enumerate as examples of this kind of synthesis the re-formation of the red-blood pigment (the hæmoglobin), the formation of the different proteins from simpler substances, and the production of fat from carbohydrates. This last-mentioned process, the formation of fat from carbohydrates, is also an example of reduction processes which occur to a considerable extent in the animal body.

Formerly the view was generally accepted that ANIMAL OXIDATION takes place in the fluids, while to-day we are of the opinion, derived from the investigations of PFLÜGER and his pupils,[2] that it is connected with the form-elements and the tissues. The question as to how this oxidation in the form-elements is induced and how it proceeds cannot be answered with certainty.

When a substance is oxidized by neutral oxygen at the ordinary temperature or at the temperature of the body, the substance is said to be easily oxidized or autoöxidized, and the process is considered as a direct oxidation or autooxidation. As the oxygen of the inspired air, and that of the blood, is neutral molecular oxygen, the old assumption that ozone occurs in the organism has now been discarded for several reasons. On the other hand, the chief groups of organic nutritives, carbohydrates, fat, and proteins, the last two forming the chief mass of the animal body, are not autoöxidizable substances. They are on the contrary bradoxidizable (TRAUBE) or dysoxidizable bodies.

---

[1] Berzelius, Lehrb. d. Chemie, übersetzt von Wöhler, 4, p. 356, Abt. 1, Dresden, 1831.

[2] Pflüger, Pflüger's Archiv, 6 and 10; Finkler, *ibid.*, 10 and 14; Oertmann, *ibid.*, 14 and 15; Hoppe-Seyler, *ibid.*, 7.

They are nearly indifferent to neutral oxygen, and it is therefore a question how an oxidation of these and other dysoxidizable bodies is possible in the animal body.

In explanation it is very generally admitted that the oxygen is made active and this causes a secondary oxidation. It is generally conceded that in autoöxidation a cleavage of neutral oxygen takes place. The autoöxidizable substance splits the oxygen molecule and combines with one of the oxygen atoms, while the other free atom as active oxygen may oxidize the dysoxidizable substances simultaneously present. Such a subordinate oxidation is called an indirect or secondary oxidation. The explanation of animal oxidations has been attempted in different ways by the supposition that the oxygen is made active and thus produces secondary oxidation.

The cause of the animal oxidation is considered, by PFLÜGER and several other investigators, to be dependent upon the special constitution of the protoplasmic proteins or the living protoplasmic substance. This investigator calls the proteins outside of the organism, or those which occur in the animal fluids, "non-living proteins," and considers them to be somewhat different from those occurring in living protoplasm. The latter are called "living proteins" (PFLÜGER), "active proteins" (LOEW), or "biogens" (VERWORN). The living protoplasmic molecule differs from the ordinary non-living protein by being more unstable and therefore having a greater inclination towards intramolecular changes of the atoms. The reason for these greater intramolecular movements PFLÜGER ascribes to the presence of cyanogen, and LATHAM attributes it to the presence of a chain of cyanalcohols in the protein molecule. VERWORN,[1] on the contrary, claims an intramolecular introduction of oxygen into a large hypothetical protoplasmic molecule, the "biogen molecule," which is supposed to contain a nitrogen or an iron complex as an oxygen receptor or carrier, and a side-chain of aldehydic character like that of the carbohydrates, as an oxidizable group.

According to LOEW,[2] who bases his claim upon special investigations and numerous toxicological observations, the unstability of the active proteid molecule is due to the simultaneous presence of aldehyde and unstable amino groups. These occur separated from each other in the active proteins, and when they combine the protoplasm dies, the molecule being changed into a stable condition, i.e., into dead protein. It is also a fact that all substances which react with aldehyde and unstable amino groups are poisonous to the living cells.

---

[1] Pflüger, Pflüger's Archiv, **10**; Latham, Brit. Med. Journal, 1886; Verworn, Die Biogenhypothese, Jena, 1903.

[2] Loew and Bokorny, Pflüger's Archiv, **25**; O. Loew, *ibid.*, **30**; and specially O. Loew. The Energy of Living Protoplasm, London, 1896.

Loew has also shown, in conjunction with **Bokorny**, that in many plants a very unstable reserve-protein substance occurs, which to a certain extent occupies an intermediate position between protein and organized living substance.

The explanation as to the oxidation process differs entirely according to the conception of the structure of the unstable protoplasmic molecule. If the living protoplasmic protein is not, like protein in the ordinary sense, indifferent to neutral oxygen, we can admit of a cleavage of the oxygen molecule by this change. The protein would be itself oxidized, while on the other hand a secondary oxidation of other difficultly oxidizable substances could be brought about by the oxygen atoms set free.

Another very widely diffused view exists in regard to the origin of the activity of the oxygen, namely, that by the decomposition processes in the tissues, reducing substances are formed which split the neutral oxygen molecule, uniting with one oxygen atom and setting the other free.

The formation of reducing substances during fermentation and putrefaction is generally known. The butyric fermentation of dextrose in which hydrogen is set free—$C_6H_{12}O_6 = C_4H_8O_2 + 2CO_2 + 2H_2$—is an example of this kind. Another example is the appearance of nitrates in consequence of an oxidation of nitrogen in cases of putrefaction, which process is ordinarily explained by the statement that reducing, easily oxidizable bodies are formed which split oxygen molecules, liberating oxygen atoms which afterward oxidize the nitrogen. It is assumed also that the cells of the animal tissues and organs have the power, like these lower organisms which produce fermentation and putrefaction, of causing splitting processes in which easily oxidizable substances, perhaps also nascent hydrogen (Hoppe-Seyler [1]), are produced.

In accordance with what has been stated above on the oxidations of the animal body, primarily a cleavage of the organic constituents of the body takes place with the formation of readily oxidizable substances. The oxidation of these latter produces an activation of the oxygen and hence may also cause a secondary oxidation of dysoxidizable substances. The products formed by these splittings and oxidations may perhaps in part be burned within the body without undergoing further cleavage, but more probably they must first undergo a further cleavage and then succumb to consecutive oxidations, until after repeated cleavages and oxidations the final products of metabolism are formed.

An activation of the oxygen may be produced according to O. Nasse[2] by a hydroxylization of the constituents of the protoplasm with the splitting off of molecules of water. If benzaldehyde is shaken with water and air, an oxidation of the benzaldehyde into benzoic acid takes place, while oxidizable substances present at the same time may also be oxidized. The simultaneous presence of potassium iodide and starch or tincture of guaiacum causes a blue coloration because the hydroxyl (OH) takes the place of the hydrogen in the aldehyde group, and these two hydrogen atoms, one derived from the aldehyde and the other from the water, have a splitting action on the molecular oxygen. NASSE and RÖSING[1] have also found that certain varieties of protein have the property of being hydroxylized in the presence of water. According to NASSE a whole series of oxidations in the animal body may be accounted for by the oxygen atoms set free in hydroxylization similar to that of benzaldehyde. In opposition to this view we must remark that the oxidation of benzaldehyde to benzoic acid may also take place in other ways, thus by the intermediary formation of a peroxide (see BAEYER and VILLIGER; ENGLER and WEISSBERG[2]).

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[1] Pflüger's Archiv, 12.

[2] O. Nasse, Rostocker Zeitung, No. 534, 1891, and No. 363, 1895.

By quantitative methods VAN'T HOFF and his pupils[3] have shown that molecular oxygen can be divided in two parts by certain autoöxidation processes. One of these unites with the autoöxidizer and the other with a body simultaneously present but not directly oxidizable, which, according to the suggestion of ENGLER,[4] is called the acceptor. VAN'T HOFF claims that the oxygen molecule dissociates at ordinary temperatures into minimum quantities of positively and negatively charged oxygen atoms, the ions of similar charge uniting with the autoöxidizable substance, while the remaining ions oxidize the acceptor. Such a division of the oxygen into two halves has also been shown by other investigators, such as MANCHOT, ENGLER, and his collaborators.[5] These investigators nevertheless consider that autoöxidation takes place in another way, namely, by the formation first of peroxides by the taking up of oxygen molecules.

TRAUBE[6] has also expressed a similar view. According to him, in autoöxidation we have to deal, in the first place, not with a cleavage of the oxygen, but with a splitting of water in which the hydroxyl groups of the water combine with the oxidizable substance, while the hydrogen atoms set free on the decomposition of the water unite with the neutral oxygen, forming hydrogen peroxide, which may naturally also have an oxidizing action.

$$A + 2H_2O + O_2 = A(OH)_2 + H_2O_2.$$

---

[1] E. Rösing, Untersuchungen über die Oxydation von Eiweiss in Gegenwart von Schwefel. Inaug. Dissert. Rostock, 1891.

[2] Baeyer and Villiger, Ber. d. d. chem. Gesellsch., **33**; Engler and Weissberg, *ibid.*, **33.**

[3] van't Hoff, Zeitschr. f. physikal. Chem., **16**; Jorissen, Ber. d. d. chem. Gesellsch., **30**, and Zeitschr. f. physikal. Chem., **22**; Ewan, *ibid.*, **16.**

[4] Ber. d. d. chem. Gesellsch., **33.**

[5] Manchot, Über freiwillige Oxydation, Leipzig, 1900; Engler and Weissberg, Ber. d. d. chem. Gesellsch., **33**; Engler and Frankenstein, *ibid.*, **34.**

[6] Ber. d. d. chem. Gesellsch., **15, 18, 19, 22,** and **26.**

According to the view of **Engler** and his collaborators, which corresponds in great measure with those of **Bach** and of **Manchot**,[1] at least in the simplest cases ("direct autoöxidation" according to **Engler**), the oxygen molecules unite with the activating body (A), forming a peroxide-like substance which can give up one of the two oxygen atoms to an acceptor (B):

$$A+O_2=AO_2 \quad \text{and} \quad AO_2+B=AO+BO.$$

If this is so, still we do not know to what extent such peroxides are formed in the oxidation in the living cell. The possibility of a production of peroxides, and also of hydrogen peroxide, in animal oxidation is still generally admitted, and **Chodat** and **Bach**[2] have indeed been able to show a peroxide formation in plants. Still, if hydrogen peroxide were formed in such oxidations it would have no further physiological importance, according to **Loew**, because the animal and plant cells contain special enzymes, called by him *catalases*, which quickly decompose the hydrogen peroxide with the production of molecular oxygen. According to **Loew**[3] the physiological importance of the catalases is to protect the cell from hydrogen peroxide, which acts as a protoplasmic poison.

Loew,[4] who has also opposed the view as to the oxygen becoming active with the setting free of oxygen atoms, has sought for the reason of the oxidations in the unstable properties of the protoplasmic proteins. The active movement of the atoms within the active protein molecule is transmitted to the oxygen and to the oxidizable substance, and when the dissolution of the molecule has proceeded to a certain point the oxidation occurs by virtue of the chemical affinity. The reason for this unstable condition of living protein molecules has already been given above.

Schmiedeberg,[5] who also denies the supposition that the oxygen becomes active, is of the view that the tissues by the mediation of the oxidations do not increase the oxidizing activity of the oxygen, but more probably act on the oxidizing substances, making them more susceptible to oxidation.

All the views presented thus far assume a continuous oxidation of the primary active substance. The view has also been suggested that animal oxidation may be brought about by oxygen-carriers, i.e., by bodies which, according to the older views, without being oxidized themselves, act in an analogous manner to the nitric oxide in the manufacture of sulphuric acid by alternately taking up and giving off oxygen in the oxidation of dysoxidizable bodies. TRAUBE has for a long time explained the oxidations of the animal body in this way, and he calls these questionable oxygen-carriers *oxidation ferments.*[1]

---

[1] Engler and Wild, Ber. d. d. chem. Gesellsch., **30**; Bach, Le Moniteur scientifique, 1897, and Compt. rend., 124; Manchot, l. c.

[2] Ber. d. d. chem. Gesellsch., **35** u. **36**.

[3] Loew, U. S. Dept. of Agriculture, Rep. 68, 1901, and Ber. d. d. chem. Gesellsch., **35**; in regard to the opposed views see Chodat and Bach, l. c., and Kastle and Loevenhart, Amer. Chem. Journ., **29**.

[4] O. Loew, The Energy of Living Protoplasm, London, 1896.

[5] Arch. f. exp. Path. u. Pharm., **14**.

It has also been positively proved by the researches of JAQUET, SALKOWSKI, SPITZER, RÖHMANN, ABELOUS and BIARNÈS, BERTRAND, BOURQUELOT, DE REY-PAILHADE, MEDWEDEW, POHL, JACOBY, CHODAT and BACH,[2] and others that in the blood and different tissues of the animal body, as also in plant-cells, substances occur which have the property of causing certain oxidations and are therefore called oxidation ferments or *oxidases.* The nature and mode of action of these bodies will be discussed elsewhere in this volume, hence it will be sufficient here to state that in general two different groups of oxidation ferments are recognized. The ferments of the first group, called primary or direct oxidases or simply *oxidases,* transfer the oxygen of the air directly to other bodies. Those of the second group, the indirect oxidases or *peroxidases,* are active only in the presence of a peroxide, as they set oxygen free from these latter by decomposition.

The many different views in regard to the oxidation processes show us strikingly how little is positively known about these processes. There is no doubt that the animal body possesses in the so-called oxidation ferments important means of bringing about oxidative decomposition of various substances, and the occurrence of numerous intermediary metabolic products in the animal body teaches us that the oxidation of the constituents of the body is not instantaneous and sudden, but takes place step by step, and hand in hand with cleavages. Most investigators are agreed that these decompositions are similar to certain oxidations studied by DRECHSEL[3] outside the animal body, where oxidations and reductions alternate in quick succession. The views are divided in regard to the manner and origin of this cooperative action.[4]

The oxidations in the animal body have long been designated as a combustion, and such a conception is easily reconcilable with the above-mentioned views. In combustion in the ordinary sense, as, for example, the burning of wood or oil, we must not forget that the substances themselves do not combine with oxygen. It is only after the action of heat has decomposed these bodies to a certain degree that the oxidation of the products of such decomposition takes place and is accompanied by the phenomenon of light.

---

[1] M. Traube, Theorie der Fermentwirkungen, Berlin, 1858.

[2] Jaquet, Arch. f. exp. Path. u. Pharm., **29**; Salkowski, Centralbl. f. d. med. Wissensch., 1892 and 1894, and Virchow's Arch., **147**; Spitzer, Pflüger's Archiv, **60** and **67**; Spitzer and Röhmann, Ber. d. deutsch. chem. Gesellsch., **28**; Abelous et Biarnès, Arch. de physiol. (5), **7**, **8**, and **9**, and Compt. rend. Soc. biol., **46**; Bertrand, Arch. de physiol. (5), **8**, **9**, and Compt. rend., **122**, **123**, **124**; Bourquelot, Compt. rend. Soc. biol., **48**, and Compt. rend., **123**; Jacoby, Ergebnisse der Physiologie, Jahrg. I, Abt. 1, which contains the literature of the subject; Chodat and Bach, l. c.

[3] Journ. f. prakt. Chem. (N. F.), **22**, **29**, **38**, and Festschrift für C. Ludwig, 1887.

[4] See M. Nencki, Arch. des sciences biol. de St. Pétersbourg, **1**, 483; Abelous and Aloy, Compt. rend., **136**, **137**; Kastle and Elvove, Amer. Chem. Journ., **31**; Underhill and Closson, Amer. Journ. of Physiol., **13**.

The essential source of heat and mechanical work developed in the organism is to be found in the oxidations. Chemical energy is transformed into the above-mentioned forms of energy in cleavage processes, where complicated chemical compounds are reduced to simpler ones, and therefore the atoms change from an unstable to a stabler equilibrium, and stronger chemical affinities are satisfied. The animal body may also have a source of energy in the cleavage processes which are not dependent on the presence of free oxygen. The processes taking place in the living muscle are an example of this kind. A removed muscle, which gives off no oxygen when in a vacuum, may, as HERMANN[1] has shown, work, at least for a time, in an atmosphere devoid of oxygen, and give off carbon dioxide at the same time.

Cleavage processes which are accompanied by a decomposition of water and then a taking up of its constituents are called *hydrolytic cleavages.* These cleavages, which play an important rôle within the animal body, and which are most frequently met with in the processes of digestion, are exemplified by the transformation of starch into sugar and the splitting of neutral fats into the corresponding fatty acids and glycerine:

$$\underset{\text{Tristearin}}{C_3H_5(C_{18}H_{35}O_2)_3}+3H_2O=\underset{\text{Glycerine}}{C_3H_5(OH)_3}+\underset{\text{Stearic acid}}{3(C_{18}H_{36}O_2)}.$$

As a rule the hydrolytic cleavage processes as they occur in the animal body may be performed outside of it by means of higher temperatures with or without the simultaneous action of acids or alkalies. Considering the two above-mentioned examples, we know that starch is converted into sugar when it is boiled with dilute acids, and also that the fats are split into fatty acids and glycerine on heating them with caustic alkalies or by the action of superheated steam. The heat or the chemical reagents which are used for the performance of these reactions would cause immediate death if applied to the living body. Consequently the animal organism must have other means at its disposal which act similarly, but in such a manner that they may work without endangering the life or normal constitution of the tissues. Such means have been recognized in the so-called *unorganized ferments* or *enzymes.*

---

[1] Untersuch. über den Stoffwechsel der Menschen, Berlin, 1867.

Alcoholic fermentation and other processes of fermentation and putrefaction are dependent upon the presence of living organisms, ferment fungi, and splitting fungi of different kinds. The ordinary view, according to the researches of PASTEUR, is that these processes are to be considered as phases of the life of these organisms. The name *organized ferments* or *ferments* has been given to such micro-organisms, of which ordinary yeast is an example. However, the same name has also been given to certain bodies or mixtures of bodies of unknown organic origin which are products of the chemical work within the cell, and which after they are removed from the cell still have their characteristic action. Such bodies—for example, malt diastase, rennin, and the digestive ferments—are capable in the very smallest quantity of causing a decomposition or cleavage in very considerable quantities of other substances, without entering into permanent chemical combination with the decomposed body or with any of the cleavage or decomposition products. These formless or *unorganized ferments* are generally called *enzymes*, according to KÜHNE.

A ferment in a more restricted sense is therefore a living being, while an enzyme is a product of chemical processes in the cell, a product which has an individuality even without the cell, and which may be active when separated from the cell. The splitting of invert-sugar into carbon dioxide and alcohol by fermentation is a fermentative process closely connected with the life of the yeast. The inversion of cane-sugar is, on the contrary, an enzymotic process caused by one of the bodies or a mixture of bodies formed by the living ferment, which can be severed from this ferment, and still remain active even after the death of the latter. Consequently ferments and enzymes are capable of manifesting a different behavior towards certain chemical reagents. Thus there exist a number of substances, among which we may mention arsenious acid, phenol, toluene, salicylic acid, boracic acid, sodium fluoride, chloroform, ether, and protoplasmic poisons, which in certain concentration kill ferments, but which do not noticeably impair the action of the enzymes.

The above view as to the difference between ferments and enzymes has lately been essentially shaken by the researches of E. BUCHNER[1] and his pupils. He has been able to obtain from beer-yeast, by grinding and strong pressure, a cell fluid rich in protein which when introduced into a solution of a fermentable sugar caused a violent fermentation. The objections raised from several sides that the fluid expressed still contained dissolved living cell substance has been so successfully answered by BUCHNER and his collaborators that there is at present no question but that alcoholic fermentation is caused by a special enzyme called *zymase* which is formed in the yeast-cell.

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[1] E. Buchner, Ber. d. deutsch. chem. Gesellsch., **30** and **31**; E. Buchner and Rapp, *ibid.*, **31**, **32**, **34**; H. Buchner, Sitzungsber. d. Gesellsch. f. Morphol. u. Physiol. in München, **13**, 1897, part 1, which also contains the discussion on this topic. See also E. and H. Buchner and M. Hahn, Die Zymasegärung, München, 1903; Stavenhagen, Ber. d. deutsch. chem. Gesellsch., **30**; Albert and Buchner, *ibid.*, **33**; Buchner, *ibid.*, **33**; Albert, *ibid.*, **33**; Albert, Buchner, and Rapp, *ibid.*, **35**; in regard to the opposed views see Macfadyen, Morris, and Rowland, *ibid.*, **33**; Wroblewski, Centralbl. f. Physiol., **13**, and Journ. f. prakt. Chem. (N. F.), **64**.

As from the yeast-cell so also from other lower organisms, indeed from the lactic-acid bacilli and beer-vinegar bacteria, we have recently been able to isolate enzymes (E. BUCHNER and MEISENHEIMER, HERZOG [1]) which produce the specific fermentative action of the mother organism. The question whether there exist ferment processes which, in PASTEUR'S sense, are the result of the biological phenomena connected with the metabolism of the micro-organism and which we can directly identify with the life processes, is very difficult to answer; hence for the present we have no foundation for a sharp differentiation between the organized ferments and enzymes. The metabolic processes of the living organisms which we recognize as fermentation phenomena must as a rule be ascribed to enzymes acting within the cell. If such processes are closely connected with the life of the cell, then this is explained in part by the fact that this special enzyme is produced only by living cells and in part by the fact that it cannot be separated from the living cells or that it is readily destroyed on the death of the cell.

All enzymes are organic substances formed in the cells, whose chemical nature has unfortunately not been established at the present time. Thus far no enzyme has been prepared in a pure state with positiveness. The enzymes are considered as protein bodies by many investigators, but this opinion has not sufficient foundation, and is disputed at least for certain enzymes. It is indeed true that the enzymes isolated by certain investigators acted like genuine protein bodies; but it is uncertain whether or not the products isolated in these instances were pure enzymes or were composed of enzymes contaminated with proteins.

The enzymes may be extracted from the cells and tissues by means of water or glycerine, especially by the latter, which forms very stable solutions and hence is extensively used as a means of extracting them. The enzymes, generally speaking, do not appear to be diffusible, and BREDIG [2] has given several reasons, which will be given later, for considering them not as true solutions but rather colloidal ones. The enzymes are also absorbed by other colloids and are carried down by fine precipitates, and this property is extensively taken advantage of in their preparation.[3]

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[1] E. Buchner and J. Meisenheimer, Ber. d. d. chem. Gesellsch., **36**; Herzog, Zeitschr. f. physiol. Chem., **37**.

[2] Anorganische Fermente, Leipzig, 1901.

[3] See Brücke, Wien. Sitzungsber., **43**, 1861.

The manner of combination of the enzymes with the colloids has not been explained and is no doubt not the same in all cases.[1] They are precipitated from their solutions by alcohol. All enzymes lose their specific action on boiling their aqueous solutions, and this is generally considered as an important criterion as to the ferment nature of a body. The continued heating of their solutions above 80° C. generally destroys the enzymes. In the dry state, however, certain enzymes may be heated to 100° or indeed to 150–160° C. without losing their activity. Light can also destroy enzymes in watery solution, as shown with malt diastase (EMMERLING) and chymosin (EMMERLING, SCHMIDT-NIELSEN).[2]

The action of the enzymes may be markedly influenced by external conditions. The reaction of the liquid is of special importance. Certain enzymes act only in acid; others, and the majority, on the contrary, act only in neutral or alkaline liquids. Certain of them act in very faintly acid as well as in neutral or alkaline solutions, but best at a specific reaction. They are all destroyed by concentrated mineral acids and alkalies. The temperature exercises also a very important influence. In general the activity of enzymes increases to a certain limit with the temperature. This optimum is not always the same, but, as shown by TAMMANN, depends, like the destructive action of high temperatures, essentially upon the quantity of enzyme and other conditions. The products of the enzymotic processes exercise a retarding influence in proportion as they accumulate, and indeed the enzymotic process may thereby be entirely stopped. In such cases of "false equilibrium" (BREDIG) we may, as shown by TAMMANN,[3] often start the reaction again by removing the products of the reaction, by diluting with water, by raising the temperature, by the addition of more substance, or by the addition of more of the enzyme. The addition of neutral salts and other substances of various kinds has in some cases an accelerating, and in other cases a retarding action.[4]

The velocity of the enzyme action and the final condition at the conclusion of the enzymotic processes is not only dependent upon the reaction, the temperature, and the presence of transformation products or of foreign bodies, but also upon the amount of enzyme present and the concentration of the solution. The velocity increases regularly with an increase in the amount of enzyme, but not in the same proportion with all enzymes, as it has been shown for different enzymes that they require different times for action. This will be discussed later. The concentration of the solution is also of great importance, and the result of a change of this during enzymotic action is of special importance in the study of the kinetics of enzyme reactions.

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[1] Dauwe, Hofmeister's Beiträge, **6**.

[2] Emmerling, Ber. d. d. chem. Gesellsch., **34**; Schmidt-Nielsen, Hofmeister's Beiträge, **5**.

[3] The work of Tammann may be found in Zeitschr. f. physiol. Chem., **16**, and Zeitschr. f. physikal. Chem., **3** and **18**.

[4] See Fermi and Pernossi, Zeitschr. f. Hygiene, **18**; also in regard to the enzymes in general see C. Oppenheimer, Die Fermente, 2. Aufl., 1903.

We have no characteristic reactions for all enzymes in general, but each enzyme is characterized by its specific action and by the conditions under which it operates. Of special importance is, first, the fact that the enzymes do not form permanent chemical combinations in definite proportions by weight with the bodies upon which they act, or their decomposition products; and, secondly, that an insignificantly small amount of the enzyme can decompose a relatively enormous amount of substance. For instance, 1 part of invertin can invert 100,000 parts of cane-sugar (O'SULLIVAN and THOMPSON [1]), and 1 part of chymosin can in a short time decompose more than 400,000 parts of casein (HAMMARSTEN [2]). This does not exclude the possibility of a primary, but temporary, combination of the enzymes with the substances acted upon. Such an assumption is, indeed, substantiated by the work of HANRIOT, HENRI, ARMSTRONG,[3] and others, while, according to OPPENHEIMER,[4] we can represent ferment action as consisting of a first phase where combination of the enzyme and the substance occurs, and a second phase where after this combination a chemical decomposition of the substance occurs according to the laws of catalysis. This view coincides best with the specificity of enzyme action.

The specific action of the enzymes is of special importance, as one and the same enzyme acts only upon one substance or a definite group of substances. Their action seems to be entirely dependent upon the stereometric construction of the substance acted upon, and we may assume that the enzyme attacks only specially arranged stereometric atomic groups, where the enzyme fits the substance in a manner similar to a key fitting a lock (E. FISCHER). E. FISCHER [5] has given a positive proof for the great importance of a different stereometric configuration by his investigations upon the artificially prepared series of stereoisomeric glucosides which he calls α- and β-glucosides. The enzymes of yeast infusions act only upon the glucosides of the α-series, while emulsin, on the contrary, acts only upon those of the β-series.

Of especially great importance for a deeper insight into the manner of enzyme action, we must mention the investigations which have been carried on recently on the relationship of inorganic catalyzers to the enzymes, which have thrown light upon the correspondence between catalysis and enzyme action. The catalyzers, like the enzymes or their derivatives, are not found in the final products of the reaction, they are not used up in the process, and the quantity of the active substance proportionate to the quantity of substance transformed is infinitesimally small in enzyme action as well as in catalysis. In both, the reaction velocity also seems to be independent of the quantity of the active substance added, and this indicates that the enzyme action is not to be considered as the starting of a reaction which would not of itself take place, but rather as an acceleration of a slowly proceeding, often not noticeable, chemical change. According to this conception enzyme action comes in line with catalysis, for, according to OSTWALD,[1] bodies are called catalyzers which by their presence cause a change in the reaction velocity of chemical processes, and indeed positive or negative, according as they produce acceleration or retardation. The striking correspondence between enzymes and inorganic catalyzers has been shown especially by BREDIG and his collaborators, V. BERNEK, IKEDA, and REINDERS,[2] by their very important investigations.

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[1] O'Sullivan and Thompson, Journ. of Chem. Soc., 57.

[2] See Maly's Jahresbericht, 7.

[3] Hanriot, Compt. rend., 132; Henri, Lois générales de l'action des diastases, Paris, 1903, and Arch. di Fisiol., 1 and 2; Armstrong, Proc. Roy. Soc. London, 73.

[4] Die Fermente, 2. Aufl., 1903, p. 66.

[5] Zeitschr. f. physiol. Chem., 26.

BREDIG has been able to prepare colloidal solutions of platinum, gold, and silver by allowing the electric arc to play between two poles of the respective metal beneath water. These solutions of colloidal metals, metallic sols, show in their activity and the dependence of this activity upon external influences, and especially in their destruction by poisons, such strong resemblance to the enzymes that BREDIG has indeed called them inorganic ferments.

Still it is nevertheless true that the manner of action of catalyzers has not been explained, and we must be careful not to draw too positive conclusions from the remarkable correspondence of the manner of action of metallic sols and certain ferments. In studying the action of enzymes one is repeatedly struck with the marked deviation from the laws of reaction underlying inorganic catalyzers,[3] and this has called forth a series of hypotheses and attempts at explanation, which on account of space cannot be entered into, but we must refer the reader to special works on the subject. On the other hand, we must not forget that the enzymes are not pure substances, but are habitually mixtures whose action may be modified by an apparently insignificant admixture, and for this reason the study of the mode of action is made very difficult. Although the question as to whether enzymes follow the same laws as the inorganic catalyzers is still an open one, nevertheless we know that in a great many regards the enzymes correspond with catalyzers. The comparison of these two has opened up in the study of enzyme action new points of elucidation and attack which have been very fruitful in result, and which have no doubt helped very much in the explanation of these difficult questions.

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[1] Grundriss d. allgemein. Chemie, 3. Aufl., 1899.

[2] See Bredig, Anorganische Fermente, Leipzig, 1901, and also Die Elemente d. chemischen Kinetik, etc., Ergebnisse der Physiologie, Jahrg. I, Abt. 1, 1902.

[3] See Brown and Glendinning, Proc. Chem. Soc. 18, 1902; Tammann, Zeitschr. f. physikal. Chem., 3 and 18, and Zeitschr. f. physiol. Chem., 16; Henri, Zeitschr. f. physikal. Chem., 39, and Lois générales, etc. See also the work of H. Euler, Zeitschr. f. physiol. Chem., 45.

It is not within the scope of this book to enter more in detail into the various theories of catalysis. Still it seems important at least to present in a few words one of these, namely, that of H. v. EULER.[1] This theory explains the mode of action of enzymes and the inorganic catalyzers by assuming an increased concentration of the active molecules producing the reaction, i.e., by increasing the ions occurring in the solution.

The action of enzymes presupposes the presence of water, and the best-studied enzymotic processes, the hydrolyses, are comparable with the action of acids and bases, i.e., the action of $\overset{+}{H}$ and $\overset{-}{HO}$ ions. In the hydrolyses by enzymes an activation of the water takes place, and the assumption that the enzymes act by an increased concentration of the $\overset{+}{H}$ and $\overset{-}{HO}$ ions, which bring about the reaction, seems to be attractive. The enzymes acting analogously to mineral acids have been assumed, according to this view, to be producers of $\overset{+}{H}$ ions, which strongly accelerate cleavages which would otherwise take place very slowly or with immeasurable velocity. This explanation may, as developed by FRIEDENTHAL,[2] be applied to the oxidation enzymes, the oxidases, which will be treated of later. Water is also imperative for animal oxidations, and the reaction of the fluid is in this case also important because oxidations are regularly accelerated by an alkaline reaction, i.e., by the presence of $\overset{-}{HO}$ ions. We can, according to FRIEDENTHAL, consider the oxidases as producers of hydroxyl ions, just as we can consider pepsin as a producer of hydrogen ions. It is apparent that this view, that the oxidases are producers of hydroxyl ions, is in harmony with the previously mentioned views of TRAUBE and NASSE,[3] that the hydroxyl ions of the water combine with the oxidizable substance.

An enzyme is an organic substance formed in an animal or plant cell, which is destroyed by heating its aqueous solution and which acts like the catalyzers, but only upon certain bodies. Some restriction must be put to this, as the cells do not always produce a complete enzyme, but oftener only the mother-substance thereof. These mother-substances of the enzymes are called *proenzymes* or *zymogens*. The zymogens are under certain conditions converted into enzymes, and in certain cases this is brought about by the special action of bodies called *kinases*, which have been little studied (see Chapters VI and IX).

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[1] Zeitschr. f. physikal. Chem., 36.

[2] Salkowski's Festschrift, 1904.

[3] See pp. 5 and 6.

The enzymes are, as above mentioned, not characterized by chemical reactions in the ordinary sense, but by their action. From this standpoint most of the enzymes which have been studied can be divided into two chief groups, namely, those enzymes having a *hydrolytic* action and those having an *oxidizing* action.

Among the hydrolytic enzymes we must mention in the first place the *proteolytic* or those which dissolve proteid, whose representatives, pepsin and trypsin, occur in the animal kingdom; the *lipolytic* or fat-splitting; and the *amylolytic* or *diastatic* enzymes, which act upon the starches. In this group we must include the *invertases*, which split the disaccharides into simpler forms of sugar. In close relationship to these enzymes we may mention the *glucoside-splitting* enzymes, which occur especially in the higher plants. Among the hydrolytic enzymes of the animal kingdom we must also include *arginase*, which splits arginine into urea and ornithine; the two desaminating enzymes *adenase* and *guanase*, which convert the two bodies adenine and guanine, with the splitting off of ammonia, into hypoxanthine and xanthine respectively; and the hippuric-acid-splitting *histozym* and the urea-splitting *urease*. The *proteid-coagulating* enzymes, *chymosin* or casein-coagulating, and *thrombin* or blood-coagulating enzyme, belong to a special though not clearly defined group.

The best-known and most carefully studied enzyme actions, the hydrolyses, are exothermal processes, and therefore the sum of the new products produced has a lower heat of combustion than the original substance. Now, as syntheses are generally endothermal reactions, i.e., are processes requiring a taking up of heat where external energy must be supplied before they take place, and also as the enzymes are not a source of energy, it used to be generally considered that the enzymes could not bring about any syntheses. This view is nevertheless untenable, and it has also been shown that enzymotic hydrolyses may be reversible processes which produce syntheses. CROFT HILL has shown that maltase, which, as is well known, has a splitting action upon maltose, also has the power of regenerating from glucose two isomeric bioses, one a new body called *revertose* and another which is probably maltose (see also EMMERLING [1]). E. FISCHER and E. F. ARMSTRONG [2] were able to obtain a dissaccharide, *isolactose*, from galactose and glucose by means of kephir lactase. HANRIOT,[3] KASTLE and **Loevenhart**[1] have shown that the lipases can bring about syntheses, and finally **Emmerling**[2] has been able to synthesize amygdalin from mandelic-acid-nitrile glucoside and glucose by means of the yeast maltase. According to **Abelous** and **Ribaut**[3] the pig and horse kidneys contain an enzyme which produces hippuric acid from benzyl alcohol and glycocoll. These investigators are of the opinion that the benzyl alcohol is first oxidized to benzoic acid and then that the synthesis is brought about by the aid of the energy set free in this process. There is more and more tendency to accept the view that the intracellular enzymes, which will be discussed later, are of importance for the syntheses in the animal body.

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[1] Hill, Ber. d. d. chem Gesellsch., **34**, and Transactions Chem. Society, 1903, **83**; Emmerling, Ber. d. d. chem. Gesellsch., **34**.

[2] Ber. d. d. chem. Gesellsch., **35**.

[3] Compt. rend., **132**.

The second group of enzymes include the so-called *oxidation ferments*, which, as above remarked, are recognized as of great importance in bringing about oxidations in the animal body. These enzymes do not all act in the same way, and correspondingly we differentiate between direct oxidases or oxidases proper, and indirect oxidases or peroxidases. Certain investigators include among the oxidation enzymes still a third group, the catalases, which split peroxides into hydrogen and oxygen.

Those enzymes which transfer oxygen to other bodies and oxidize them are called *oxidases* or direct oxidases. *Peroxidases* or indirect oxidases are, on the contrary, enzymes having an oxidizing action only in the presence of hydroperoxides or another peroxide, as they decompose the peroxide and bring about oxidation by the oxygen set free. Correspondingly the oxidases turn tincture of guaiacum blue directly, while the peroxidases only have this action in the presence of a peroxide. The *catalases* do not give any reaction with guaiacum either directly or indirectly in the presence of peroxides.

According to the investigations of **Bach** and **Chodat**[4] the conditions are otherwise. According to the observations they have made upon plants, there exist no oxidases and what has been described under this name is only a mixture of oxygenases and peroxidases. The *oxygenases* are of a protein nature, contain manganese or iron, and are converted into peroxides after taking up oxygen. These peroxides themselves have only a slight oxidizing power but are made active by the peroxidases. The peroxidases, which do not have the slightest oxidizing power in the absence of peroxides, are not proteins. In oxidation, according to the hypothesis of **Bach** and **Chodat**, the molecular oxygen is first converted by the oxygenase into peroxide. This peroxide is activated by the peroxidase and then has strong oxidizing power. The oxidizing power of the so-called direct oxidases is brought about by a combined action of the oxygenases and peroxidases.

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[1] Amer. Chem. Journ., **24**.

[2] Ber. d. d. chem. Gesellsch., **34**, 3810.

[3] Compt. rend. Soc. biol., **52**; Maly's Jahresber., **30**.

[4] Biochem. Centralbl., **1**, pp. 417 and 457.

The chemical nature of the oxidation enzymes is still unknown, and the statements on this subject are very contradictory. Certain oxidases are supposed to be nucleoproteids (SPITZER), others globulins (ABELOUS and BIARNÈS), and still others, like the liver aldehydase (JACOBY) and laccase (BERTRAND), are of a non-protein nature. The materials upon which the oxidation enzymes act may also be very different from each other. Thus the oxidases studied by RÖHMANN and SPITZER may by synthetical oxidation produce indophenol from α-naphthol and p-phenylenediamine in the presence of alkali. The salicylase or aldehydase detected in the liver and many other organs oxidizes many aldehydes to their corresponding acids, but does not give the indophenol reaction. The laccase isolated by BERTRAND from the juice of the lac-tree has an oxidizing action upon polyhydric p-phenols, such as hydroquinone, but not upon tyrosine. The bodies called tyrosinases, first found by BERTRAND [1] in certain fungi and later also found by BIEDERMANN, V. FÜRTH, and SCHNEIDER in the animal kingdom, have, on the contrary, an action upon tyrosine, converting it into homogentisic acid (GÖNNERMANN [2]) or other colored compounds. Another oxidase occurring in the liver and spleen, and called xanthine oxidase by BURIAN, has the property, as shown by SPITZER, WIENER, SCHITTENHELM, and BURIAN,[3] of transforming xanthine and hypoxanthine into uric acid by oxidation.

The oxidases and peroxidases as well as the catalases occur very widely distributed in the animal and plant kingdoms.

Like other enzymes, the oxidation enzymes show also a pronounced specificity; thus a certain oxidase, for instance laccase, oxidizes only certain substances and not others. This behavior, which is difficult of explanation according to the common hypotheses as to the action of oxidation enzymes, indicates, according to MEDWEDEW,[4] that in the oxidation the active substances do not act upon the oxygen, but rather upon the substance to be oxidized. We cannot at present give any statement as to the extent of action of the oxidation enzymes in the oxidations of the animal body, and it is still a question whether in all cases where oxidation enzymes have been claimed to have been found we were actually dealing with enzymes.

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[1] In regard to the work of the various authors cited, see foot-note, p. 8.

[2] Biedermann, Pflüger's Archiv, **72**; v. Fürth and Schneider, Hofmeister's Beiträge, **1**; Gönnermann, Pflüger's Archiv, **82**.

[3] Spitzer, Pflüger's Archiv, **76**; Wiener, Arch. f. exp. Path. u. Pharm., **42**; Schittenhelm, Zeitschr. f. physiol. Chem., **42** and **43**; Burian, *ibid.*, **43**.

[4] Pflüger's Archiv, **81**.

In investigations with hydroperoxides and vegetable peroxidases Bach and Chodat[1] found that peroxides and peroxidases always took part in the reaction in constant proportions, and that the peroxidases were quickly used up, which certainly does not indicate that these bodies have an enzymotic nature. Aso[2] has also shown that in certain cases where an apparent oxidase action was present very probably we were dealing only with nitrites which were present; and finally, attention must be called to the fact that manganese or iron, sometimes in considerable amounts, has been found in many oxidases. As manganous and ferrous salts are active as catalyzers in certain other oxidations, so also in certain cases important rôles as oxygen-carriers have been ascribed to these metals, for instance in laccase, which contains manganese (Bertrand), and the oxidases containing iron (Spitzer's nucleoproteid). Manchot[3] by his work on the autooxidation of ferrous sulphate has called attention to the apparently great importance of iron for physiological oxidations, and the work of Trillat,[4] who has prepared colloidal solutions of protein-manganese which had great similarity to oxidase solutions, is also of special interest.

Our knowledge of the reducing enzymes,[5] the so-called *reductases* or *hydrogenases*, is even still more meagre. Certain investigators claim that the so-called *philothions*, which develop hydrogen sulphide in the presence of sulphur and water, belong to this group, while others, on the contrary, do not accept this view and consider the enzymotic nature of the philothions as doubtful.[6] There is no doubt that reductions occur to a great extent in the animal body and often hand in hand with oxidations; nevertheless the question as to how far special reduction enzymes take part in these reductions is still an open one. According to Abelous and Aloy[7] we have indeed enzymes that have an oxidizing as well as a reducing action, for they obtain the oxygen necessary for the oxidation of one body by removing it from another substance through reduction.

The property of decomposing hydrogen peroxide has been observed with many enzymes, but this property does not belong to them,[8] depending rather upon another enzyme, a catalase, which often adheres to other enzymes as an impurity. The catalases were first closely studied by O. LOEW,[1] and he has investigated two different catalases—the α- and β-catalase. The first, which is not soluble in water, is a nucleoproteid, while the other, β-catalase, is soluble in water and is a proteose.

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[1] Ber. d. d. chem. Gesellsch., **37**.

[2] Beihefte zum botan. Centralbl., **18**.

[3] Zeitschr. f. anorg. Chem., **27**.

[4] Compt. rend., **137**, **138**.

[5] Abelous and Gerard, Compt. rend., **129**; Pozzi-Escot, Bull. Soc. chim. (3), **27**.

[6] De Rey-Pailhade, Recherches expér. sur le Philothion, etc., Paris (G. Masson), 1891, and Nouvelles recherches sur le Philothion, Paris (G. Masson), 1892; Pozzi-Escot, l. c., and Chem. Centralbl., 1904, 1, S. 1645; Chodat and Bach, Ber. d. d. chem. Gesellsch., **36**; Abelous and Ribaut, Compt. rend., **137**, and Bull. Soc. chim., Paris (3), **31**.

[7] Compt. rend., **136**, **137**, and **138**.

[8] See Al. Schmidt, Zur Blutlehre, Leipzig, 1892; Jacobson, Zeitschr. f. physiol. Chem., **16**.

The catalases, whose action consists in decomposing hydrogen peroxide into oxygen and hydrogen, occur widely diffused in the animal and plant kingdoms. According to L. LIEBERMANN[2] the fatty tissues among the animal structures seem to be richest in catalases, an observation which has been substantiated and developed by EULER.[3] The liver, kidneys, and spleen are relatively rich in catalases, while the brain and muscles are poor therein; still the proportions vary somewhat for different varieties of animals.[4] As has long been known, the blood also contains a catalase, which has been called *hæmase* by SENTER.[5]

The physiological importance of the catalases is still unknown. According to LOEW[6] they have the function of destroying the hydrogen peroxide, which occurs perhaps as an intermediary product in oxidations and which has a destructive action as protoplasmic poison; but this assumption is disputed by EULER[7] and others. EULER calls attention to the parallelism which exists between the fat-splitting and the peroxide-splitting action of plant and animal extracts and claims that the lipolytic extracts have the property of decomposing hydrogen peroxide.

The glycolytic or sugar-destroying enzyme, which occurs in the blood and tissues and which takes part in the decomposition of the sugars, stands in close relationship to the oxidases. We will discuss this enzyme in a following chapter in speaking of glycosuria and the question of diabetes, and it is here sufficient to remark that certain investigators, like SPITZER, consider this enzyme as an oxidase, while others, on the contrary, consider the decomposition of the sugar in the tissues to be a process analogous to alcoholic fermentation.

Alcoholic fermentation by means of yeast or zymase is not an oxidation in the ordinary sense, where the sugar takes up free oxygen. It is rather an internal oxidation where a part of the molecule is oxidized at the cost of another part, and finally a destruction into alcohol and carbon dioxide takes place. According to the recent investigations of BUCHNER and MEISENHEIMER, STOKLASA, and MAZÉ,[1] we are dealing here with the united action of two enzymes, one the *lactolase* (STOKLASA) or *lactacidase* (BUCHNER and MEISENHEIMER), which converts the sugar into lactic acid, while the other, the *zymase* (BUCHNER and MEISENHEIMER) or *alcoholase* (STOKLASA), splits the lactic acid into alcohol and carbon dioxide. According to several investigators, the sugar passes into lactic acid, with methylglyoxal, $CH_3.CO.CHO$, as an intermediary body.

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[1] U. S. Dept. of Agriculture, Rep. 68, Washington, 1901, and Ber. d. d. chem. Gesellsch., 35.

[2] Pflüger's Arch., 104.

[3] Hofmeister's Beiträge, 7, which also gives the references to the literature.

[4] See Battelli and Stern, Compt. rend., 138; Battelli and Haliff, Compt. rend. Soc. biol., 57.

[5] Senter, Zeitschr. f. physikal. Chem., 44, also A. Jolles and Oppenheim, Virchow's Arch., 180; Ville and Moitesnier, Bull. Soc. chim. (3), 29; A. Rosenbaum, Salkowski's Festschrift, 1904.

[6] See foot-note, 3, p. 7.

[7] Hofmeister's Beiträge, 7.

STOKLASA and his collaborators[2] believe that an alcoholic fermentation by means of a zymase or perhaps a mixture of the two above-mentioned enzymes, lactolase and alcoholase, also takes place in animal tissues. Objections to these investigations have been made by several experimenters who claim essentially that in these cases we are dealing only with the action of micro-organisms.[3] HAMMARSTEN considers that the views of STOKLASA and his collaborators have not been disproved, and one cannot exclude the possibility that an alcoholic fermentation may also occur in the animal tissues in anaerobic respiration.

The enzymes, in certain instances, may also act upon one another, and as an example of this kind of action we may mention BUCHNER's zymase, which can be destroyed by the proteolytic enzyme of the yeast-cells. Pepsin, which has a destructive action upon diastases and especially upon trypsin, is another example. Of special interest is the action of the anti-enzymes upon the enzymes, which consists in retarding or arresting the specific action of the enzyme by a corresponding anti-body. This subject will be discussed later.

Unfortunately considerable confusion exists in the nomenclature of the enzymes. In most cases the enzyme is named after the substance upon which it acts, thus amylase, lipase, arginase, urease; in other cases according to its action, thus oxidase, reductase; while in certain cases the products produced are the basis for the name, thus alcoholase, lactacidase, glucase. In order to obtain a clear and concise nomenclature of the enzymes v. LIPPMANN[4] has suggested that we construct the name of the enzyme out of two words, one of which represents the substance acted upon by the enzyme, while the second is the important or chief product produced by the enzyme. Thus maltoglucase is an enzyme which produces *d*-glucose from maltose, amylmaltase one that forms maltose from starch (amylum), etc.

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[1] Buchner and Meisenheimer, Ber. d. d. chem. Gesellsch., **37** and **38**; Stoklasa, Ber. d. d. botan. Gesellsch., **22**, pp. 358 and 460; Mazé, Compt. rend., **138**.

[2] Hofmeister's Beiträge, **3**; Centralbl. f. Physiol., **16**, **17**, **18**; Ber. d. d. chem. Gesellsch., **38**; see also Čzerný, *ibid.*, **36**, with Jelinek, Šimaček, and Vitek, Pflüger's Arch., **101**.

[3] See the work of O. Cohnheim, Zeitschr. f. physiol. Chem., **39**, **42**, **43**; Battelli, Compt. rend., **137**; Portier, Compt. rend. Soc. biol., **57**.

[4] Ber. d. d. chem. Gesellsch., **36**.

Many enzymes are secreted by the cells as such or as proenzymes. They act outside of the cells in which they were formed, or they act after having been transformed into the enzyme, and hence are called secretion enzymes or extracellular enzymes.

Besides these extracellular enzymes we also have another group which act within the cells, hence are intracellular and therefore are called intracellular enzymes or endoenzymes. Numerous enzymes besides the yeast zymase belong to this group, and seemingly also oxidases and enzymes having hydrolytic action. The best studied of this group are the proteolytic enzymes, which were first observed by SALKOWSKI and his pupils, and which bring about the self-digestion or autodigestion of organs in the absence of micro-organisms. This *autodigestion* has been the subject of numerous investigations, principally by the HOFMEISTER school and especially by JACOBY.[1] The latter has given the name *autolysis* to the process, and he has shown that the enzymes taking part in this action do not come from the digestive tract and are not pepsin or trypsin taken up by the cells. In autolysis we are not only dealing with a proteolysis, but several other processes occur, such as the splitting of fats and carbohydrates, oxidations and reductions, and perhaps also syntheses.

We therefore generally designate as autolysis all the enzyme actions which take place in removed organs or fluids without the aid of micro-organisms, but it must not be forgotten that autolytic processes may also occur *intra vitam* under certain conditions. The combined action of various enzymes in autolysis also explains to us why, as especially shown by LEVENE and by JONES,[2] the products obtained by the hydrolytic cleavage of an organ by means of an acid are somewhat different from those products produced on autolysis.

It is at present impossible to state what part autolytic processes take in life under physiological conditions, and we can have only conjectures on this subject. In the autolysis of a removed organ or of one through which the blood is not flowing, the conditions in many ways are quite different from the conditions in life. The products which appear after weeks or months of autolysis, sometimes in very small quantities, do not give any clue to the nature of the processes, and conclusions must be drawn very carefully from these results.

The post-mortem autolyses, as far as studied, are chiefly proteolyses, but we must not forget that the enzymes taking part are in many cases most active in acid reaction, while they have only a weak action or are inactive in neutral or alkaline reaction. The observations of LANE-CLAYPON and SCHRYVER,[1] that the autolysis of the liver and kidney begins only after a latent period of from two to four hours subsequent to the removal of the organ, are also of interest. It is possible that this is due to the fact that the enzymes are first formed from the proenzymes after the death of the organ, or perhaps certain conditions tending to retard the enzymotic action are removed. Recent investigations of WIENER[2] show that the post-mortem formation of acid is the important factor in this. It is difficult to judge of the importance of the autolytically active proteolytic enzymes for the physiological life of the cells, but there does not seem to be any doubt as to the importance of these enzymes in pathological conditions.

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[1] A complete summary of the literature of intracellular enzymes and autolysis may be found in Jacoby, Über die Bedeutung der intrazellulären Fermente, etc., Ergebnisse der Physiologie, Jahrg. I, Abt. 1, 1902.

[2] Levene, Amer. Journ. of Physiol., **11** and **12**, and Zeitschr. f. physiol. Chem., **41**; W. Jones, *ibid.* **42**.

The changes of the liver and blood in acute phosphorus intoxication and in acute yellow atrophy of the liver, where we find in the urine the enzymotic decomposition products of the proteins, are examples of an *intra vitam* autolysis which is considered by some as an abnormal rise in the physiological autolysis. Another example is the solution of pneumonic infiltrations by the enzymes of the migrated and inclosed leucocytes as studied by FR. MÜLLER,[3] and this is at the same time an example of *heterolysis*, i.e., of a solution or a destruction in an organ by enzymes not belonging therein but introduced from without. An autolysis, although not very marked, occurs in those organs or parts of organs which have not been normally nourished because of a disturbance in the circulation, and they are gradually consumed by this action. The part injured undergoes solution, while the healthy part remains unattacked. By this solvent action as well as by the formation of bactericidal bodies, as observed by CONRADI,[4] and of antitoxins (BLUM [5]) by means of autolysis, we can consider this autolysis as a remedy and perhaps also as a protective agent for the animal body.

For the present it is impossible to judge of the importance of the enzymes active in autolysis for physiological conditions, but this does not exclude the possibility that in normal cell life the enzymes play a very important rôle. Numerous observations show this to be true, and we tend more and more toward the view that the chemical transformations in the living cells are brought about by enzymes and that these latter are to be considered as the chemical tools of the cells (HOFMEISTER and others [6]).

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[1] Journ. of Physiol., **31**.

[2] Centralbl. f. Physiol., **19**, p. 349.

[3] Verhandl. d. naturforsch. Gesellsch. zu Basel, 1901. See also O. Simon, Deutsch. Arch. f. klin. Med., 1901.

[4] Hofmeister's Beiträge, **1**.

[5] *Ibid.*, **5**, p. 142.

[6] F. Hofmeister, Die chemische Organisation der Zelle, Braunschweig, 1901.

As above stated, the chemical processes in animals and plants do not stand in opposition to each other; they offer differences indeed, but still they are of the same kind from a qualitative standpoint. PFLÜGER says that there exists a blood-relationship between all living cells of the animal and vegetable kingdoms, and that they originate from the same root. The animal body is a complex of cells, hence study of the chemical processes must not only be made upon higher plants but also upon unicellular organisms in order that we get a proper explanation of the chemical processes in the animal organism. Although a biochemical study of the micro-organisms is very important, we must bear in mind also the important rôle played by such organisms in animal life, chiefly as exciters of disease; hence the study of the conditions of life of these micro-organisms and the products produced by them must be of infinite importance in their chemical investigation.

The products produced by micro-organisms may be of very different kinds. Among the substances produced in the decomposition of animal fluids and tissues by putrefactive organisms we find those having a basic nature. To this class belong the cadaver alkaloids called *ptomaines*, first found by SELMI in human cadavers and then specially studied by BRIEGER and GAUTIER.[1] Certain of these are poisonous, designated as *toxines*, while the others are non-poisonous. They all belong to the aliphatic compounds and generally do not contain oxygen. As an example of these basic substances we must mention the two diamines, *cadaverine* or pentamethylenediamine, $C_5H_{14}N_2$, and *putrescine* or tetramethylenediamine, $C_4H_{12}N_2$, which have awakened special interest because they occur in the contents of the intestine and in the urine in certain pathological conditions, especially in cholera and cystinuria.[2] Among the bodies produced by putrefaction, the bacterial poison *sepsine*, $C_5H_{14}N_2O_2$, recently isolated by E. FAUST,[3] is of especially great interest because to this substance we ascribe the characteristic toxic action of putrefactive masses. Sepsine was prepared by FAUST as a crystalline sulphate which on repeated evaporation of its solution was readily converted into cadaverine sulphate.

Those substances of basic nature which are incessantly and regularly produced as products of the decomposition of the protein substances in the living organism, and which therefore are to be considered as products of the physiological metabolism, have been called *leucomaines* by GAUTIER in contradistinction to the ptomaines and toxines produced by micro-organisms. These bodies, to which belong several well-known animal extractives, were isolated by **Gautier**[1] from animal tissues such as the muscles. The hitherto known leucomaines, of which a few are poisonous in small amounts, belong to the choline, the uric acid, and the creatinine groups.

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[1] Selmi, Sulle ptomaine od alcaloidi cadaverici e loro importanza in tossicologia, Bologna, 1878; Ber. d. deutsch. chem. Gesellsch., **11**, Correspond. by H. Schiff; Brieger, Ueber Ptomaine, Parts 1, 2, and 3, Berlin, 1885–1886; A. Gautier, Traité de chimie appliquée à la physiologie, **2**, 1873, and Compt. rend., **94**.

[2] See Brieger, Berlin. klin. Wochenschr., 1887; Baumann and Udransky, Zeitschr. f. physiol. Chem., **13** and **15**; Brieger and Stadthagen, Berlin. klin. Wochenschr., 1889.

[3] Arch. f. exp. Path. u. Pharm., **51**.

The leucomaines are considered as being of certain importance in causing disease. It has been contended that when these bodies accumulate on account of an incomplete excretion or oxidation in the system, an auto-intoxication may be produced (**Bouchard** and others[2]).

Of especially great interest are the toxines which are found in the higher plants and animals, like the jequirity-bean and castor-seed, in the poison of snakes and spiders, in blood-serum, etc., and particularly those produced by pathogenic micro-organisms which have an unmistakable relationship to the enzymes. A closer study of these various bodies, lysines, agglutinines, toxines, etc., as well as of the antitoxines and the theory of immunity, does not lie within the scope of this work, and although the subject is of the greatest importance, it cannot be treated here. We can only call attention to one similarity between many toxines and enzymes, and this is important in connection with what we have already stated in regard to the enzymes. As by the repeated introduction of a toxine into an animal body we can excite a formation of the corresponding antitoxine, so, as first shown by **Morgenroth**,[3] it is also possible, by the increasing introduction of an enzyme (rennin, for example), to produce an antienzyme (an antirennin) in the body. Similar antienzymes have been produced in several other cases, but this is not surprising, as this is only a special case of the general immunity theory, according to which the animal body has the power of making foreign substances non-destructive by reaction products formed by the body.

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[1] Bull. Soc. chim., 43, and A. Gautier, Sur les alcaloïdes dérivés de la destruction bactérienne ou physiologique des tissus animaux, Paris, 1886.

[2] Bouchard, Leçons sur les auto-intoxications dans les maladies, Paris, 1887. See also the various text-books of clinical medicine.

[3] Centralbl. f. Bakteriol. u. Parisitenkunde, 26.

CHAPTER II.

THE PROTEIN SUBSTANCES.

The chief mass of the organic constituents of animal tissues consists of amorphous, nitrogenized, very complex bodies of high molecular weight. These bodies, which are either proteids in a special sense or bodies nearly related thereto, take first rank among the organic constituents of the animal body on account of their great abundance. For this reason they are classed together in a special group which has received the name *protein group* (from πρωτεύο, I am the first, or take the first place). The bodies belonging to these several groups are called *protein substances*, although in a few cases the protein bodies in a special sense are designated by the same name.

The several *protein substances*[1] contain *carbon*, *hydrogen*, *nitrogen*, and *oxygen*. The majority contain also *sulphur*, a few *phosphorus*, and a few also *iron*. *Copper*, *chlorine*, *iodine*, and *bromine* have been found in some few cases. On heating the protein substances they gradually decompose, producing a strong odor of burnt horn or wool. At the same time they produce inflammable gases, water, carbon dioxide, ammonia, and nitrogenized bases, besides many other substances, and leave a large quantity of carbon. On hydrolytic cleavage they all yield, besides nitrogenous basic substances, especially large amounts of α-monamino-acids of different kinds.

The nitrogen occurs in the protein bodies in various forms, and this is also revealed in the division of the nitrogen among the cleavage products. On boiling with dilute mineral acids we obtain (1) so-called amide nitrogen, which is readily split off as ammonia; (2) a guanidine residue which is combined with diaminovalerianic acid as arginine and which has also been called the urea-forming group; (3) basic nitrogen or diamino-acid nitrogen, which is precipitated by phosphotungstic acid as basic products (to which also the guanidine residue of arginine belongs); (4) monamino-acid nitrogen;

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[1] See "Eiweisskörper," Ladenburg's Handwörterbuch der Chemie, 3, 534–589, which gives a very complete summary of the literature of protein substances up to 1885. The more recent literature up to the year 1903 may be found in O. Cohnheim, Chemie der Eiweisskörper, Braunschweig, 1904. See also Mann, Chemistry of the Proteids, London, 1906.

and (5) the nitrogen in variable amounts which appears as humus-like melanoidins, which seem to be of only secondary formation as products of elaboration.

The quantitative division of the total nitrogen between the above five groups is different in the various protein substances, and moreover cannot be given with certainty, because of the above-mentioned melanoidin formation and the errors in the methods used.[1] The following gives at least an approximate idea of this division.[2] The loosely combined so-called amide nitrogen seems to be entirely absent in the protamines. In the gelatines we find 1-2 per cent, and 5-10 per cent in other animal protein substances; in the plant gluten-proteids, 13-20 per cent of the total nitrogen is amide nitrogen. The guanidine nitrogen may amount in the protamines to 22-44 per cent of the total nitrogen, in the histones to 12-13 per cent, in the gelatines about 8 per cent, and in the other protein bodies about 2-5 per cent. As basic nitrogen precipitable by phosphotungstic acid (including the guanidine residue) we find 35-88 per cent in the protamines, 35-42.5 per cent in the histones, 15-25 per cent in the other animal protein substances, 5-14 per cent in zein and the gluten proteid, and up to 37 per cent in the plant globulins. The chief quantity of the nitrogen, 55-76 per cent, occurs, with the exception of the protamines, as the monamino-acid groups. The results for the melanoidin nitrogen vary so considerably that they will not be mentioned.

From the above results it follows that the nitrogen of most protein bodies exists in such combination that the chief quantity appears in the cleavage products as amino-compounds on hydrolytic cleavage by acids. By the action of nitrous acid upon proteins only a very small part, 1-2 per cent, of the nitrogen is evolved,[3] which seems to indicate that NH_2 groups exist only to a slight extent in protein substances. This assumption does not have sufficient foundation, for, according to LEVITES,[4] the quantity of amide nitrogen is not diminished by the action of nitrous acid upon the protein substances. In view of several observations, it is generally admitted that the amino-groups occurring in the cleavage products exist in the original protein substance chiefly as imino-groups.

The sulphur occurs in the different protein bodies in very different amounts. Certain of them, such as the protamines and apparently also certain bacterial proteids,[1] are free from sulphur; some, such as gelatine and elastin, are very poor in sulphur; while others, especially horn substances, are relatively rich in sulphur. On hydrolytic cleavage with mineral acids, the sulphur of the protein substances is regularly, at least in part, split off as cystine (K. MÖRNER) or, with bodies poorer in sulphur, as cystein (EMBDEN), but this, according to MÖRNER and PATTEN, is a secondary formation. From certain protein substances α-thiolactic acid (SUTER, FRIEDMANN, FRÄNKEL), which MÖRNER claims is also produced secondarily, mercaptans and sulphuretted hydrogen (SIEBER and SCHOUBENKO, RUBNER), and a body having the odor of ethyl sulphide (DRECHSEL) have been obtained.[2]

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[1] See the work of Hausmann, Zeitschr. f. physiol. Chem., 27 and 29; Henderson, *ibid.*, 27; Kossel and Kutscher, *ibid.*, 30; Kutscher, *ibid.*, 31, 38; Hart, *ibid.*, 33; Gümbel, Hofmeister's Beiträge, 5; Rothera, *ibid.*

[2] See the works given in foot-note 1 and Blum, Zeitschr. f. physiol. Chem., 30; Kossel, Ber. d. d. chem. Gesellsch., 34, 3214; Hofmeister, Ergebnisse der Physiol., Jahrg. I, Abt. 1, 759, which also contains the literature; Osborne and Harris, Journ. Amer. Chem. Soc., 25; and Gümbel, l. c.

[3] See C. Paal, Ber. d. d. chem. Gesellsch., 29; H. Schiff, *ibid.*, 1354; O. Loew, Chemiker Zeitung, 1896; and O. Nasse, Pflüger's Arch., 6.

[4] Levites, Zeitschr. f. physiol. Chem., 43.

A part of the sulphur separates as potassium or sodium sulphide on boiling with caustic potash or soda, and may be detected by lead acetate and quantitatively determined (FLEITMANN, DANILEWSKY, KRÜGER, FR. SCHULZ, OSBORNE, K. MÖRNER[3]). What remains can be detected only after fusing with potassium nitrate and sodium carbonate and testing for sulphates. The ratio between the sulphur split off by alkali and that not split off is different in various proteins. No conclusions can be drawn from this in regard to the number of forms of combination which the sulphur has in the protein molecule. As shown by K. MÖRNER, only about three-fourths of the sulphur in cystine can be split off by alkali, and the same is true for the cystine-yielding complex of the protein substances. If the quantity of lead-blackening sulphur in a protein body be multiplied by $\frac{4}{3}$, we obtain the quantity corresponding to the cystine sulphur in the body. By such calculation MÖRNER found in certain bodies, such as horn substance, seralbumin and serglobulin, that the quantity of cystine sulphur and total sulphur were identical, and therefore we have no reason for considering the sulphur in these bodies as existing in more than one form of combination. In other proteins, such as fibrinogen and ovalbumin, on the contrary, only one-half or one-third of the sulphur appeared as cystine sulphur.

According to RAIKOW[4] keratin-like proteins split off sulphur dioxide on treatment with phosphoric acid at ordinary temperatures; hence it follows that a part of the sulphur in the proteins, especially in the keratins, exists in direct combination with oxygen and probably combined as in the sulphites.

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[1] See Nencki and Schaffer, Journ. f. prakt. Chem. (N. F.), 20, and M. Nencki, Ber. d. d. chem. Gesellsch., 17.

[2] K. Mörner, Zeitschr. f. physiol. Chem., 28, 34, and 42; Patten, *ibid.*, 39; Embden, *ibid.*, 32; Suter, *ibid.*, 20; Friedmann, Hofmeister's Beiträge, 3; Sieber and Schoubenko, Archiv d. sciences biol. de St. Pétersbourg, 1; Rubner, Arch. f. Hygiene, 19; Drechsel, Centralbl. f. Physiol., 10, 529; Fränkel, Sitzungsber. d. Wien. Akad., 112, II *b*, 1903

[3] Fleitmann, Annal. der Chem. und Pharm., 66; Danilewsky, Zeitschr. f. physiol. Chem., 7; Krüger, Pflüger's Archiv, 43; F. Schulz, Zeitschr. f. physiol. Chem., 25; Osborne, Connecticut Agric. Expt. Station Report 1900; Mörner, l. c.

[4] See Biochem. Centralbl., 4, p. 353.

The constitution of the protein bodies is still unknown, although the great advances made in the last few years have brought us essentially closer to the elucidation of the question. In studying the constitution of the protein bodies they have been broken up in various ways into simpler portions, and the methods used for this purpose have been of different kinds. In such decompositions, for which the proteids in the true sense have been primarily used, especially those that can be prepared in the crystalline form, first large atomic complexes—proteoses and peptones—are obtained which still have protein characteristics, and these then suffer further decomposition until finally we obtain simpler, generally crystalline, or at least well characterized end products.

On heating protein with barium hydrate and water in sealed tubes to 150-250° C. SCHÜTZENBERGER[1] obtained a mixture of products among which were ammonia, carbon dioxide, oxalic acid, acetic acid, and, as chief product, a mixture of amino-acids. The conclusion he drew from this experiment, that the proteid is a complex ureide or oxamide, cannot be considered for several reasons.[2]

On fusing proteins with caustic alkali we obtain ammonia, methyl mercaptan, and other volatile products; also leucine, from which then volatile fatty acids, such as acetic acid, valerianic acid, and also butyric acid are obtained, and also tyrosine, from which latter phenol, indol, and skatol are produced. As to the products prepared by hydrolytic cleavage with mineral acids we have a number of investigations by various experimenters, especially HLASIWETZ and HABERMANN, RITTHAUSEN and KREUSLER, E. SCHULZE and his collaborators, DRECHSEL, SIEGFRIED, R. COHN, KOSSEL and his pupils, K. MÖRNER, ABDERHALDEN, SKRAUP, and recently E. FISCHER and his collaborators.[3] The chief products thus obtained are monamino-acids, such as glycocoll, alanine, aminovalerianic acid, leucine, tyrosine, phenylaminopropionic acid, aspartic and glutamic acids, cysteine and its sulphide cystine; the so-called hexone bases, lysine, arginine, and histidine, of which the first two are diamino-acids; oxymonamino-acids, such as serine, oxyaminosuccinic acid, and oxyaminosuberic acid; oxydiamino-acids, such as oxydiaminosuberic acid, oxydiaminosebacic acid, diaminotrioxydodecanoic acid, caseanic and caseinic acids; α-pyrrolidine and oxypyrrolidine carboxylic acids; indolaminopropionic acid; sulphuretted hydrogen, ethyl sulphide, leucinimide, ammonia, and melanoidins,[1] which latter seem to be secondary condensation products.

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[1] Annal. de chim. et phys. (5), 16, and Bull. Soc. chim., 23 and 24.

[2] See Habermann and Ehrenfeld, Zeitschr. f. physiol. Chem., 30.

[3] In regard to the literature see O. Cohnheim, Chemie der Eiweisskörper, Braunschweig, 1904, and F. Hofmeister, Ergebnisse der Physiologie, Jahrg. I, Abt. 1, 759, 1902; E. Fischer, Untersuchungen über Animosäuren, Polypeptide und Proteïne (1899-1906), Berlin, 1906; also Mann, Chemistry of the Proteids, London, 1906. See also special references.

The proteins can be split into a large number of bodies by the proteolytic enzymes, and these will be presented later. In the first place proteoses and peptones are produced, also an abundance of monamino-acids of different kinds, hexone bases, tryptophane (proteinochromogen), which is indolaminopropionic acid, and finally oxyphenylethylamine, diamines, and a little ammonia and other substances.

A great many substances are produced in the putrefaction of proteins. First the same bodies as are formed in the decomposition by means of proteolytic enzymes are produced, and then a further decomposition occurs with the formation of a large number of bodies belonging in part to the aliphatic and in part to the aromatic and heterocyclic series. Of the first series we have ammonium salts of volatile fatty acids, such as caproic, valerianic, and butyric acids, also succinic acid, carbon dioxide, methane, hydrogen, sulphuretted hydrogen, methyl mercaptan, and others. The ptomaines also belong to these products, and are probably in part formed by very different chemical processes, or even syntheses.

E. SALKOWSKI divides the putrefactive products of the aromatic and heterocyclic series into three groups: (*a*) the phenol group, to which tyrosine, the aromatic oxyacids, phenol, and cresol belong; (*b*) the phenyl group, including phenylacetic acid and phenylpropionic acid; and lastly (*c*) the indol group, which includes indol, skatol, skatolacetic acid, and skatolcarboxylic acid. These various products are formed during putrefaction with access of air. NENCKI and BOVET[2] obtained only *p*-oxyphenylpropionic acid, phenylpropionic acid, and skatolacetic acid on the putrefaction of proteins by anaerobic schizomycetes in the absence of oxygen. These three acids are produced by the action of nascent hydrogen on the corresponding amino-acids, namely, tyrosine, phenylaminopropionic acid, and skatolaminoacetic acid (indolaminopropionic acid), and according to NENCKI these three last-mentioned amino-acids exist preformed in the protein molecule.

By the moderate action of chlorine, bromine, or iodine upon proteins these halogens enter into more or less firm combination with the molecule (LOEW, BLUM, BLUM and VAUBEL, LIEBRECHT, HOPKINS and BROOK, HOFMEISTER, KURAJEFF, and others), and according to the method of procedure we can prepare derivatives having different but constant amounts of halogens (HOPKINS and PINKUS). The proteins are so changed that they do not split off sulphur on treatment with alkali, nor do they respond to MILLON's reaction, nor do they yield tyrosine as a cleavage product. This is ordinarily explained by the supposition that a substitution of hydrogen by iodine takes place in the aromatic tyrosine nucleus; but since according to Oswald the heteroproteoses, which yield only very little tyrosine, take up about the same quantity of iodine as the protoproteoses, which yield considerable tyrosine, it appears that the iodine is united to other groups besides the tyrosine-yielding atomic complex. By the action of iodine an oxidation also occurs, and Schmidt[1] has shown that a continuous splitting off of amino groups takes place. According to him phenol and *p*-cresol, cleavage products of tyrosine, besides benzoic acid, are produced by the oxidation of phenylaminopropionic acid.

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[1] See Samuely, Hofmeister's Beiträge, 2.

[2] Salkowski, Zeitschr. f. physiol. Chem., 12, 215, and 27, 302; Nencki and Bovet, Monatshefte f. Chem., 10.

By the oxidation of proteid by means of potassium permanganate Maly obtained an acid, *oxyprotosulphonic acid*, C 51.21, H 6.89, N 14.59, S 1.77, O 25.54 per cent, which is not a cleavage product, but an oxidation product in which the group SH is changed into $SO_2.OH$. This acid does not give the proper color reaction with Millon's reagent, yields no tyrosine or indol, but gives benzene on fusing with alkali. On continued oxidation Maly obtained another acid, *peroxyproteic acid*, which gives the biuret reaction, but is not precipitated by most protein precipitants. The *oxyprotein* obtained by Schulz on the oxidation of proteid by hydrogen peroxide is closely related to oxyprotosulphonic acid in composition and general characteristics, but contains lead-blackening sulphur and gives Millon's reaction. The oxyprotein is claimed to be a pure oxidation product, while in the production of oxyprotosulphonic acid Schulz claims that a cleavage takes place. According to the recent investigations of v. Fürth[2] there exist at least three different peroxyproteic acids (from casein) which differ from each other by a different division of the nitrogen in the molecule. On treatment with baryta-water we find that they split off basic complexes and oxalic-acid groups, and new bodies, the *desaminoproteic acids*, which give the biuret reaction, are produced. These acids, which on hydrolysis give benzoic acid but no diamino-acids, may be further oxidized, which is not true of the peroxyproteic acids, and yield a new group of acids, the *kyroproteic acids*, which give the biuret reaction, hold about one-half of their nitrogen (11.08 per cent total nitrogen) in acid-amide-like combination, but yield neither basic products nor benzoic acid.

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[1] Loew, Journ. f. prakt. Chem. (N. F.), **31**; Blum, Münch. med. Wochenschr., 1896; Blum and Vaubel, Journ. f. prakt. Chem. (N. F.), **57**; Liebrecht, Ber. d. deutsch. chem. Gesellsch., **30**; Hopkins and Brook, Journ. of Physiol., **22**; Hopkins and Pinkus, Ber. d. deutsch. chem. Gesellsch., **31**; Hofmeister, Zeitschr. f. physiol. Chem., **24**; Kurajeff, *ibid.*, **26**; Oswald, Hofmeister's Beiträge, **3**; C. H. L. Schmidt, Zeitschr. f. physiol. Chem., **35**, **36**, **37**.

[2] Maly, Sitzungsber. d. k. Akad. d. Wissensch. Wien, **91** and **97**. Also Monatshefte f. Chem., **6** and **9**. See also Bondzynski and Zoja, Zeitschr. f. physiol. Chem., **19**; Bernert, *ibid.*, **26**; Schulz, *ibid.*, **29**; v. Fürth, Hofmeister's Beiträge, **6**.

On the oxidation of gelatine or proteid with permanganate we obtain also oxaminic acid, oxamide, oxalic acid, oxaluric-acid amide, succinic acid, several volatile fatty acids, and guanidine, which was first shown by LOSSEN as an oxidation product (KUTSCHER, ZICKGRAF, SEEMANN, KUTSCHER and SCHENCK).[1]

On the oxidation of gelatine by ferrous sulphate and hydrogen peroxide BLUMENTHAL and NEUBERG have obtained acetone as a product, and ORGLER[2] the same from ovalbumin. JOLLES[3] claims to have obtained large quantities of urea in the oxidation of various proteins by potassium permanganate in acid solution, but this has been disputed by other investigators. On the oxidation of protein in acid liquids, volatile fatty acids, their aldehydes, nitriles and ketones, also hydrocyanic acid, benzoic acid, and other bodies, have been obtained.

Nitric acid gives various nitro-products. A melanoidin substance, *xanthomelanin*, has been obtained by v. FÜRTH.[4] HABERMANN and EHRENFELD[5] also obtained oxyglutaric acid among other products. By the action of bromine under strong pressure a number of products have been obtained: bromanil and tribromacetic acid, bromoform, leucinimide, leucine, oxalic acid, tribromaminobenzoic acid, and other bodies. With aqua regia, fumaric acid, oxalic acid, chlorazol, and other bodies are obtained. The recent investigations of HABERMANN and EHRENFELD and PANZER[6] upon the action of chlorine upon proteins and closely related products are important.

By the dry distillation of proteins we obtain a large number of decomposition products having a disagreeable burnt odor, and a porous glistening mass of carbon containing nitrogen is left as a residue. The products of distillation are partly an alkaline liquid which contains ammonium carbonate and acetate, ammonium sulphide, ammonium cyanide, an inflammable oil, and other bodies, and a brown oil which contains hydrocarbons, nitrogenized bases belonging to the aniline and pyridine series, and a number of unknown substances.

The occurrence of protein substances which contain a carbohydrate group has been known for a long time. The nature of this carbohydrate, which can be split off by acid and which may amount to as much as 35 per cent, has been explained chiefly by the investigations of FRIEDRICH MÜLLER[7] and his students. They have shown that it is always an amino-sugar and generally glucosamine. That so-called true proteids also yield a carbohydrate on hydrolytic cleavage was first shown by PAVY, using ovalbumin. The continued investigations of FR. MÜLLER, WEYDEMANN,

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[1] Lossen, Annal. d. Chem. u. Pharm., 201; Kutscher, Zeitschr. f. physiol. Chem., 32; Zickgraf, *ibid.*, 41; Seemann, *ibid.*, 44; Kutscher and Schenck, Ber. d. d. chem. Gesellsch., 37 and 38.

[2] Blumenthal and Neuberg, Deutsch. med. Wochenschr., 1901; Orgler, Hofmeister's Beiträge, 1.

[3] Zeitschr. f. physiol. Chem., 32 and 38.

[4] See Maly's Jahresber., 30, 24.

[5] Zeitschr. f. physiol. Chem., 35.

[6] Habermann and Ehrenfeld, *ibid.*, Panzer, *ibid.*, 33 and 34.

[7] Müller, Sitzungsber. d. Ges. d. Naturw. zu Marburg, 1896 and 1898, and Zeitschr. f. Biologie, 42.

SEEMANN, FRÄNKEL, HOFMEISTER, and LANGSTEIN[1] have demonstrated that in these cases the carbohydrate is also glucosamine. A carbohydrate complex, although sometimes only to a very slight amount, has also been detected in other proteins, ovoglobulin, serglobulin, seralbumin, pea-globulin, albumin of the gramineæ, yolk-proteid, and fibrin. In other proteins, on the contrary, such as edestin (of the hemp-seed) and casein, myosin, pure fibrinogen, and ovovitellin, carbohydrates have been sought for with negative results. All proteins hence do not contain a carbohydrate group, and future investigators must therefore decide whether the carbohydrate groups belong positively to the protein complex or whether they are united with the protein only as impurities. Several observations[2] show that in working with crystalline proteins a contamination with other protein substances is unfortunately not excluded, and this must not be lost sight of, especially as the quantity of carbohydrates obtained is often very small. In the present state of our knowledge we are not warranted in considering the carbohydrate groups as belonging to the carbon nucleus produced on the destruction of the real protein complex.

The previously mentioned methods used in studying the structure of the protein substances are not of the same value, but they in part substantiate each other. Of these we must mention the hydrolysis by means of boiling dilute mineral acids, or by proteolytic enzymes, as the best methods for obtaining the carbon nuclei in the protein molecule. The most important of the carbon nuclei obtained are as follows:

I. The Nuclei belonging to the Aliphatic Series.

A. *Sulphur free, but containing nitrogen:* 1. A *guanidine residue* (combined with ornithine as arginine). 2. *Monobasic monamino-acids:* Glycocoll (aminoacetic acid), alanine (aminopropionic acid), aminovalerianic acid, leucine (isobutylaminoacetic acid), and isoleucine. 3. *Bibasic monamino-acids:* Aspartic acid (aminosuccinic acid) and glutamic acid (aminoglutaric acid). 4. *Oxymonamino-acids:* serine (oxyaminopropionic acid) oxyaminosuccinic acid and oxyaminosuberic acid. 5. *Monobasic diamino-acids:* Diaminoacetic acid, ornithine (diaminovalerianic acid) and lysine (diaminocaproic acid). 6. *Oxydiamino-acids:* Oxydiaminosuberic acid, oxydiaminosebacic acid, diaminotrioxydodecanoic acid, caseanic and caseinic acids.

B. *Sulphurized:* Cysteine (aminothiolactic acid) and its sulphide cystine, thiolactic acid, mercaptans, and ethyl sulphide.

II. The Nuclei belonging to the Carbocyclic Series.

Phenylaminopropionic acid and tyrosine.

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[1] In regard to the literature on this subject see the work of Fr. Müller, Zeitschr. f. Biologie, 42, and Langstein, Ergebnisse der Physiologie, Jahrg. I, Abt. 1, 63, Zeitschr. f. physiol. Chem., 41, and Hofmeister's Beiträge, 6. See also Abderhalden, Bergell, and Dörpinghaus, Zeitschr. f. physiol. Chem., 41.

[2] See Wichmann, Zeitschr. f. physiol. Chem., 23, and N. Schulz, Die Grösse des Eiweissmoleküls, Jena, 1903, 51.

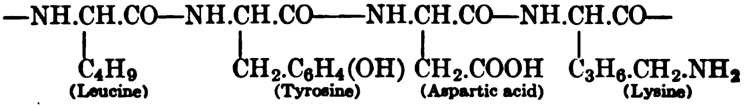
III. The Nuclei belonging to the Heterocyclic Series.

A. Of the *pyrrol group:* Pyrrolidine carboxylic acid (α-proline) and oxypyrrolidine carboxylic acid.

B. Of the *indol group:* Tryptophane or indolaminopropionic acid, from which indol and skatol are produced by putrefaction.

In regard to these carbon nuclei it must be remarked that they are not all found in every protein body thus far investigated, and also that one and the same cleavage product, such, for example, as glycocoll, leucine, tyrosine, or cystine, is obtained in very variable amounts from different protein substances. It is very difficult to say to what extent all the above-mentioned carbon nuclei exist in the protein molecule. It is not inconceivable that in the hydrolysis certain carbon nuclei may be secondarily formed from others. We cannot exclude the possibility, as suggested by LOEW,[1] that in the hydrolysis a marked atomic displacement perhaps occurs before cleavage, and for this reason two carbon nuclei, such as leucine and lysine, or tyrosine and phenylalanine, may be produced from the same atomic groupings, each according to the nature of the neighboring groups.

Even if we admit the above, still it is undoubtedly true that the chief cleavage products of the protein substances are amino-acids. EMIL FISCHER has shown that the amino-acids have the property of readily grouping together when water is split off and the amide group of one amino-acid unites with the carboxyl group of the other. In accord with this behavior we can, as HOFMEISTER[2] has explained, consider the proteins as chiefly formed by the condensation of amino-acids, where the amino-acids are united to each other by means of imino-groups according to the following scheme:



$$\begin{array}{llll}
\text{—NH.CH.CO—} & \text{NH.CH.CO——} & \text{NH.CH.CO—} & \text{NH.CH.CO—} \\
\quad | & \quad | & \quad | & \quad | \\
\quad C_4H_9 & \quad CH_2.C_6H_4(OH) & \quad CH_2.COOH & \quad C_3H_6.CH_2.NH_2 \\
\text{(Leucine)} & \text{(Tyrosine)} & \text{(Aspartic acid)} & \text{(Lysine)}
\end{array}$$

Closely connected with this conception is the question whether it is possible to prepare protein-like substances synthetically. In this connection we must mention that GRIMAUX and later also SCHÜTZENBERGER and PICKERING have been able to prepare substances which in many properties are similar to the proteins, from various amino-acids either alone or mixed with other bodies such as biuret, alloxan, xanthine, or ammonia. Of special interest are the investigations of CURTIUS and his collaborators, in which they were able to prepare synthetically the so-called *biuret base* (triglycyl-glycine ethyl ester) and subsequently many other bodies which were related to the proteins. The most important work on the chaining of amino-acids has been performed by E. FISCHER [1] and his pupils. They have prepared a large number of complex bodies called *polypeptides*, which, according to whether they contain two or more amino-acid groups united together, are called di-, tri-, tetrapeptides, etc. As examples of polypeptides we will mention—dipeptides: glycylalanine, leucyl-*l*-tyrosine, propylalanine, diaminopropionic-acid dipeptide, lysyl-lysine, histidyl-histidine; tripeptides: diglycyl-glycine, leucyl-alanyl-glycine, dileucylcystine; tetrapeptides: triglycyl-glycine, dileucyl-glycyl-glycine; pentapeptide: tetraglycyl-glycine.

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[1] Loew, Die chem. Energie d. lebenden Zellen, München, 1898, and Hofmeister's Beiträge, 1.

[2] "Über den Bau des Eiweissmoleküls." Gesellsch. deutsch. Naturforscher und Ärtze, Verhandl. 1902, and Ergebnisse der Physiologie, Jahrg. I, Abt. 1, 759.

In connection with these syntheses it is important to note that E. FISCHER and BERGELL,[2] by the decomposition of a protein substance, fibroin, by successive action of acid, proteolytic enzyme (trypsin), and baryta-water, were able to obtain a dipeptide, probably glycylalanine. LEVENE and BEATTY have obtained a dipeptide anhydride, prolineglycyl anhydride, in the tryptic digestion of gelatine, and FISCHER and ABDERHALDEN [3] have also isolated from silk fibroin a dipeptide composed of glycocoll and *l*-tyrosine. In the hydrolysis of elastin with sulphuric acid they obtained a third dipeptide, which, like the others, was an anhydride, namely, glycyl-*l*-leucine anhydride. Of the synthetically prepared polypeptides several give the biuret reaction, and in this regard, as well as their behavior towards other reagents, they are similar to the peptones, which will be discussed later. Certain polypeptides, like the biuret base (according to SCHWARZSCHILD), the glycyl-*l*-tyrosine, and alanyl-glycine, are split by trypsin, while others, like glycyl-glycine and glycylalanine, are not attacked by this me (see Chapter IX).

It is admitted that the atomic chaining in the protein consists of a union of α-amino-acids by means of the imide bonds. It is probable that also other linkings occur, and besides the above-mentioned bondage we certainly have one other, namely, the urea-forming group (the guanidine residue) united by the imide linkings with the ornithine (diaminovalerianic acid). This imide linkage is not ruptured, like that of the α-amino-acids, by trypsin, but it is by another enzyme discovered by Kossel and Dakin,[1] called arginase.

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[1] See Pickering, King's College, London, Physiol. Lab. Collect. Papers, 1897, which also cites Grimaux's work; also Journ. of Physiol., 18, and Proceed. Roy. Soc., **60**, 1897; Schützenberger, Compt. rend., **106** and **112**; Curtius, Journ. f. prakt. Chem. (N. F.), **26** and **70**, and Ber. d. d. chem. Gesellsch., **37**; Fischer and collaborators, *ibid.*, **35**, **36**, **37**, **38**, **39**, and Annal. d. Chem. u. Pharm., **340**. All the work of E. Fischer and his collaborators on this subject may be found in E. Fischer's Untersuchungen über Aminosäuren, Polypeptide und Proteïne (1899–1906), Berlin, 1906.

[2] See Biochem. Centralbl., **1**, p. 84.

[3] Levene and Beatty, Ber. d. d. chem. Gesellsch., **39**, p. 2060; Fischer and Abderhalden, *ibid.*, **39**, p. 2315.

If we consider the proteins as composed chiefly of amino-acids combined together in imide-like complexes containing also several NH_2 groups at the ends of the chains; it is easy to understand that the proteins, like the amino-acids, are amphoteric electrolytes, combining with bases as well as with acids to form salts which are strongly dissociated hydrolytically. As we must also admit of the presence in the protein molecule of a large number of COOH as well as NH_2 groups, it follows that the protein bodies may be polybasic acids, as well as polyacidic bases. In this regard the various proteins behave somewhat differently, as some of them, like the protamines, are strongly basic, while others, like casein, behave chiefly like acids, while others take a certain intermediate position. On this behavior as well as on their chemical constitution it is unfortunately impossible to base a proper classification of the protein substances. Their general properties, such as solubilities and precipitation properties, are too uncertain to aid us in the construction of a proper classification. On the other hand a classification is important, and we cannot do without one, so we will give the following systematic summary of the chief groups of the protein bodies as suggested by Hoppe-Seyler and Drechsel, which will be of some aid to us.

I. Simple Proteids or Albuminous Bodies.

Albumins	*Seralbumin*, *Lactalbumin*, and others.
Globulins	*Fibrinogen*, *Myosin*, *Serglobulins*, and others.
Nucleoalbumins	*Casein*, *Ovovitellin*, and others.
Albuminates	*Acid albuminate*, *Alkali albuminate.*
Proteoses (and Peptones).	
Coagulated Proteids	*Fibrin*, Proteids coagulated by heat, and others.
Histones (Protamines).	

II. Compound Proteids.

Hæmoglobins.	
Glucoproteids	*Mucins* and *Mucinoids*, *Amyloid*, *Ichthulin*, and others.
Nucleoproteids	*Nucleohistone*, *Cytoglobin*, and others.

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[1] Zeitschr. f. physiol. Chem., 41.

III. Albumoids or Albuminoids.

Keratins.
Elastin.
Collagen.
Reticulin.
(Fibroin, Sericin, Cornein, Spongin, Conchiolin, Byssus, and others.)

To this summary must be added that we often find in the investigations of animal fluids and tissues protein substances which do not fall in with the above scheme, or are classified only with difficulty. At the same time it must be remarked that bodies will be found which seem to rank between the different groups, hence it is very difficult to sharply divide these groups.

I. Simple Proteids or Albuminous Bodies.

The simple proteids are never-failing constituents of the animal and vegetable organisms. They are especially found in the animal body, where they form the solid constituents of the muscles and of the blood-serum, and they are so generally distributed that there are only a few animal secretions and excretions, such as the tears, the perspiration, and perhaps the urine, in which they are entirely absent or occur only in traces.

All proteids contain *carbon*, *hydrogen*, *nitrogen*, *oxygen*, and *sulphur*;[1] a few contain also *phosphorus*. *Iron* is generally found in traces in their ash, and it seems to be a regular constituent of a certain group of the albuminous bodies, namely, the nucleoalbumins. The composition of the different albuminous bodies varies a little, but the variations are within relatively close limits. For the better-studied animal proteids the following composition of the ash-free substance has been found:

C.	50.6 —54.5	per cent.
H.	6.5 — 7.3	"
N.	15.0 —17.6	"
S.	0.3 — 2.2	"
P.	0.42— 0.85	"
O.	21.50—23.50	"

The animal proteids are odorless, tasteless, and ordinarily amorphous. The crystalloid spherules (*Dotterplättchen*) occurring in the eggs of certain fishes and amphibians do not consist of pure proteids, but of proteids containing large amounts of lecithin, which seem to be combined with mineral substances. Crystalline proteids[1] have been prepared from the seeds of various plants, and crystallized animal proteids (see seralbumin and ovalbumin, Chapters VI and XIII) can be readily prepared. In the dry condition the proteids appear as white powders, or when in thin layers as yellowish, hard, transparent plates. A few are soluble in water, others only soluble in salt or faintly alkaline or acid solutions, while others are insoluble in these solvents. Solutions of proteids are optically active and turn the plane of polarized light to the left. All proteids when burned leave an ash, and it is therefore questionable whether there exists any proteid body which is soluble in water without the aid of mineral substances. Nevertheless it has not been thus far successfully proved that a native proteid body can be prepared perfectly free from mineral substances without changing its constitution or its properties.[2]

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[1] See foot-note 1, p. 28.

As previously stated, the albuminous bodies are amphoteric electrolytes and are polyacidic bases as well as polybasic acids. The base- and acid-combining powers of various proteids are different, and the maximum acid-combining power may perhaps also be used in the differentiation of the various proteids (COHNHEIM, ERB, and others).

The acid-combining power of the proteids has been studied by means of physical methods by SJÖQUIST, BUGARSKY, and LIEBERMANN and with the aid of chemical methods by SPIRO and PEMSEL, ERB, COHNHEIM and KRIEGER, v. RHORER. The methods pursued by COHNHEIM and KRIEGER consisted in precipitating the proteid from acid solution (HCl) with an alkaloid reagent (calcium phosphotungstate). The reaction took place as follows: proteid hydrochloride + calcium phosphotungstate = proteid phosphotungstate + calcium chloride. The acid remaining in the filtrate was determined, and when this quantity was subtracted from the known original amount in the proteid solution, the difference represented the acid combined with the proteid. If sodium picrate or potassium-mercuric iodide is used instead of the phosphotungstate we have, according to v. RHORER,[3] a method which is the best of all heretofore suggested.

The proteids can be salted out from their neutral solutions by neutral salts (NaCl, Na_2SO_4, $MgSO_4$, $(NH_4)_2SO_4$, and many others) in sufficient concentrations. While by other precipitants they are often changed or modified, their properties remain unchanged on salting out, and the process is reversible, as on diminishing the concentration of the salt the precipitate redissolves. SPIRO[4] has shown that we are not dealing here with the formation of a proteid combination, but rather that this is an instance of a division of a body between two solvents. The various proteids act in an essentially different manner towards the same salt, and also for one and the same proteid the behavior towards different neutral salts is different, as some cause a precipitate, while others on the contrary do not precipitate.

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[1] See Maschke, Journ. f. prakt. Chem., 74; Drechsel, *ibid.* (N. F.), 19; Grübler, *ibid.* (N. F.), 23; Ritthausen, *ibid.* (N. F.), 25; Schmiedeberg, Zeitschr. f. physiol. Chem., 1; Weyl, *ibid.*, 1.

[2] See E. Harnack, Ber. d. d. chem. Gesellsch., 22, 23, 25, and 31; Werigo, Pflüger's Archiv, 48; Bülow, *ibid.*, 58; Schulz, Die Grösse des Eiweissmoleküls, Jena, 1903.

[3] Pflüger's Arch., 90. In regard to the literature on this subject see Cohnheim, Chemie der Eiweisskörper, 2. Aufl., pp. 107–109.

[4] Hofmeister's Beiträge, 4.

According to PAULI[1] this can be explained by the fact that we have to do with ion action and that the precipitation action is the algebraic sum of the antagonistic properties. If we ascribe the precipitating action to the cations and a retarding action upon precipitation to the anions, then, according as a salt has the positive cations or the negative anions in excess, we obtain a precipitation action or not, that is, it is accelerated or retarded.

The behavior of various proteids with one and the same salt, such as $MgSO_4$ or $(NH_4)_2SO_4$, is often made use of in the isolation of the proteid, and special methods of separation are based upon fractional precipitation. HASLAM[2] has recently shown that these methods may lead to great errors and give good results only under special conditions.

The conditions are different from those of salting out, when the proteid solution is precipitated by salts of the heavy metals. Here the precipitates (often called metallic albuminates) are not true combinations in constant proportions, but are rather to be considered as loose absorption compounds of the proteid with the salt.[3] These reactions are irreversible in so far that dilution with water or removal of the salt by means of dialysis does not restore the unchanged proteid. On the other hand the precipitate, at least in certain cases, may be redissolved in an excess of the salt solution or of the proteid solution, and in this sense the process is a reversible one.

The precipitation of proteids by salts stands in close relationship to their colloidal nature. The protein bodies do not as a rule diffuse through animal membranes, or only to a very slight extent, and hence have in most cases a pronounced colloidal nature in GRAHAM's sense. Certain of them, especially the peptones and a few proteoses, which will be discussed later, seem to occupy an intermediate position between colloids and crystalloids, as their solutions are characterized by a lesser viscosity and greater diffusibility, are not readily precipitable by alcohol, not coagulable by heat, and only slightly precipitable by salts.

The solutions (or suspensions) of proteids in water, the proteid hydrosols, are converted by various means into proteid hydrogels. Of these means we must specially mention the following: flocking out with salts, precipitation with alcohol, and coagulation by means of heat.

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[1] Hofmeister's Beiträge, 3.

[2] See Cohnheim, Chemie der Eiweisskörper, 2. Aufl., 1904, pp. 144–148; Pinkus, Journ. of Physiol., 27; Pauli, Hofmeister's Beiträge, 3, p. 225; Haslam, Journ. of Physiol., 32.

[3] See Galeotti, Zeitschr. f. physiol. Chem., 40, 42, and 44.

The precipitation with alcohol is a reversible reaction, as the precipitate redissolves on subsequent dilution with water. The proteids are changed by the action of alcohol, some readily and quickly, others with difficulty and very slowly; the proteid then becomes insoluble in water and is modified.

Those proteids which occur, according to the common views, preformed in the animal fluids and tissues and which have been isolated from these by indifferent chemical means without losing their original properties are called *native proteids.* New modifications having other properties can be obtained from the native proteids by heating, by the action of various chemical reagents such as acids, alkalies, alcohol, and others, as well as by proteolytic enzymes. These new proteids are called *modified* ("*denaturierte*") *proteids,* to differentiate them from the native proteids. In the scheme given on page 36 the albumins, globulins, and nucleoalbumins belong to the native proteids, and the acid or alkali albuminates, the proteoses, the peptones, and the coagulated proteids to the modified proteids.

On heating a solution of a native proteid it is modified at a different temperature for each different proteid. With proper reaction and other favorable conditions, for instance in the presence of neutral salts, most proteids can in this way be precipitated in a solid form as coagulated proteid. The hydrosol is converted into hydrogel, but as a modification takes place, this process is irreversible. The various temperatures at which coagulation of different proteids occurs in neutral solutions containing salt have in many cases given us good means for detecting and separating proteids. The views in regard to the use of these means are somewhat divided.[1]

A modification can be brought about also by the action of acids, alkalies, or salts of the heavy metals, in certain cases by water alone, also by the action of alcohol, chloroform,[2] and ether, by violent shaking, etc.

As colloids[3] the proteids can, like other protein substances, to a more or less degree, prevent the precipitation of a colloidal metallic solution (gold solution) by means of an electrolyte (see gold equivalent according to ZSIGMONDY and SCHULZ).[1]

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[1] See Halliburton, Journ. of Physiol., **5** and **11**; Corin and Berard, Bull. de l'Acad. roy. de Belg., **15**; Haycraft and Duggan, Brit. Med. Journ., 1890, and Proc. Roy. Soc. Edin., 1889; Corin and Ansiaux, Bull. de l'Acad. roy. de Belg., **21**; L. Frédericq, Centralbl. f. Physiol., **3**; Haycraft, *ibid.*, **4**; Hewlett, Journ. of Physiol., **13**; Duclaux, Annal. Institut Pasteur, **7**. In regard to the relationship of the neutral salts to the heat coagulation of albumins see also Starke, Sitzungsber. d. Gesellsch. f. Morph. u. Physiol. in München, 1897; Pauli, Pflüger's Arch., **78**.

[2] See Salkowski, Zeitschr. f. physiol. Chem., **31**; Fr. Krüger, Zeitschr. f. Biologie, **41**; Loew and Aso, Bull. Coll. Agric. Tokio, **4**.

[3] The study of colloids and especially their changes of state is of the greatest importance for the chemistry of the proteids as well as for biochemistry in general. As the views on important points in this extensive subject are so very divergent, it is

The general reactions for the proteids are very numerous, but only the most important will be given here. To facilitate the study of these they have been divided into the two following groups:

A. Precipitation Reactions of the Proteid Bodies.

1. *Coagulation Test.* An alkaline proteid solution does not coagulate on boiling, a neutral solution only partly and incompletely, and the reaction must therefore be acid for coagulation. The neutral liquid is first boiled and then the proper amount of acid added carefully. A flocculent precipitate is formed, and if properly done the filtrate should be water-clear. If dilute acetic acid be used for this test, the liquid must first be boiled and then 1, 2, or 3 drops of acid added to each 10–15 c.c., depending on the amount of proteid present, and boiled before the addition of each drop. If dilute nitric acid be used, then to 10–15 c.c. of the previously boiled liquid 15–20 drops of the acid must be added. If too little nitric acid be added, a soluble combination of the acid and proteid is formed, which is precipitated by more acid. A proteid solution containing a small amount of salts must first be treated with about 1 per cent NaCl, since the heating test may fail, especially on using acetic acid, in the presence of only a slight amount of proteid. 2. *Behavior towards Mineral Acids at Ordinary Temperatures.* The proteids are precipitated by the three ordinary mineral acids and by metaphosphoric acid, but not by orthophosphoric acid. If nitric acid be placed in a test-tube and the proteid solution be allowed to flow gently thereon, a white opaque ring of precipitated proteid will form where the two liquids meet (HELLER's albumin test). 3. *Precipitation by Metallic Salts.* Copper sulphate, neutral and basic lead acetate (in small amounts), mercuric chloride, and other salts precipitate proteid. On this is based the use of proteids as antidotes in poisoning by metallic salts. 4. *Precipitation by Ferro- or Ferricyanide of Potassium in Acetic-acid Solution.* In these tests the relative quantities of reagent, proteid, or acid do not interfere with the delicacy of the test. 5. *Precipitation by Neutral Salts,* such as Na_2SO_4 or NaCl, when added to saturation to the liquid acidified with acetic acid or hydrochloric acid. 6. *Precipitation by Alcohol.* The solution must not be alkaline, but must be either neutral or faintly acid. It must,

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impossible to give a short review of the subject, hence we can only refer for the literature to Hamburger, Osmotischer Druck und Ionenlehre in den med. Wissenschaften, Wiesbaden, 1902–1904; H. Aron, Über organische Kolloide, Biochem. Centralbl., 3, pp. 461 and 501.

[1] Hofmeister's Beiträge, 3.

at the same time, contain a sufficient quantity of neutral salts. 7. *Precipitation by Tannic Acid* in acetic-acid solutions. The absence of neutral salts or the presence of free mineral acids may prevent the appearance of the precipitate, but after the addition of a sufficient quantity of sodium acetate the precipitate will in both cases appear. 8. *Precipitation by Phosphotungstic or Phosphomolybdic Acids* in the presence of free mineral acids. *Potassium-mercuric iodide* and *potassium-bismuth iodide* precipitate proteid solutions acidified with hydrochloric acid. 9. *Precipitation by Picric Acid* in solutions acidified by organic acids. 10. *Precipitation by Trichloracetic Acid* in 2–5 per cent solutions. 11. *Precipitation by Salicylsulphonic Acid.* The proteids are precipitated by nucleic acids, taurocholic and chondroitin-sulphuric acid in acid solutions.

B. Color Reactions for Proteid Bodies.

1. *Millon's Reaction.*[1] A solution of mercury in nitric acid containing some nitrous acid gives a precipitate with proteid solutions which at the ordinary temperature is slowly, but at the boiling-point more quickly, colored red; and the solution may also be colored a feeble or bright red. Solid albuminous bodies, when treated by this reagent, give the same coloration. This reaction, which depends on the presence of the aromatic group in the proteid, is also given by tyrosine and other monohydroxyl benzene derivatives. According to O. NASSE[2] it is best to use a solution of mercuric acetate which is treated with a few drops of a 1 per cent solution of potassium or sodium nitrite; previous to use a few drops of acetic acid are added. 2. *Xanthoproteic Reaction.* With strong nitric acid the albuminous bodies give, on heating to boiling, yellow flakes or a yellow solution. After making alkaline with ammonia or alkalies the color becomes orange-yellow. 3. *Adamkiewicz's Reaction.* If a little proteid is added to a mixture of 1 vol. concentrated sulphuric acid and 2 vols. glacial acetic acid a reddish-violet color is obtained slowly at ordinary temperatures, but more quickly on heating. According to HOPKINS and COLE[3] this reaction takes place only on using glacial acetic acid containing glyoxylic acid. According to them it is better to use a solution of glyoxylic acid, which can be readily prepared by adding sodium amalgam to a concentrated solution of oxalic acid and filtering after the discharge of the gas. A dilute aqueous

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[1] The reagent is obtained in the following way: 1 pt. mercury is dissolved in 2 pts. nitric acid (of sp. gr. 1.42), first cold and then warmed. After complete solution of the mercury add 1 volume of the solution to 2 volumes of water. Allow this to stand a few hours and decant the supernatant liquid.

[2] See O. Nasse, Sitzungsb. d. Naturforsch. Gesellsch. zu Halle, 1879, and Pflüger's Arch., **83**; see also Vaubel and Blum, Journ. f. prakt. Chem. (N. F.), **57**.

[3] Proceed. Roy. Soc., **68**.

solution of the acid or some of the solid acid is added to the proteid solution and sulphuric acid allowed to flow down the side of the test-tube, when the reddish-violet color will appear at the point of contact of the two liquids. Gelatine does not give this reaction. 4. *Biuret test.* If a proteid solution be first treated with caustic potash or soda and then a dilute copper-sulphate solution be added drop by drop, first a reddish, then a reddish-violet, and lastly a violet-blue color is obtained. 5. Proteids are soluble on heating with *concentrated hydrochloric acid,* producing a violet color, and when they are previously boiled with alcohol and then washed with ether (LIEBERMANN [1]) they give a beautiful blue solution. This blue color is due, according to COLE,[2] to a contamination of the ether with glyoxylic acid, which reacts with the tryptophane groups split off by the hydrochloric acid. 6. With *concentrated sulphuric acid* and *sugar* (in small quantities) the albuminous bodies give a beautiful red coloration. 7. With *p*-dimethylaminobenzaldehyde and concentrated sulphuric acid the proteids give a beautiful reddish-violet or deep-violet coloration (O. NEUBAUER and E. ROHDE [3]).

Many of these color reactions are obtained, as shown by SALKOWSKI,[4] by the aromatic or heterocyclic cleavage products of the proteids. MILLON'S reaction is given only by the substances of the phenol group; the XANTHOPROTEIC reaction by the phenol group and skatol or skatolcarbonic acid. LIEBERMANN'S reaction depends, according to COLE, upon the skatol (indol) group, and the reactions with sulphuric acid and sugar (COLE) and with dimethylaminobenzaldehyde (ROHDE) are also caused by this group. ADAMKIEWICZ'S reaction is given only by the bodies of the indol group. The biuret reaction is not only given by protein substances but also by many other bodies. According to H. SCHIFF [5] this reaction occurs with those bodies containing amino groups, $CONH_2$, $CSNH_2$, $C(NH)NH_2$ or also CH_2NH_2, united either directly by their carbon atoms or by means of a third carbon or nitrogen atom. As examples of such bodies we can mention several diamines or aminoamides, such as oxamide, biuret, glycinamide, α- and β-aminobutyramide, aspartic-acid amide, etc., although we are not clear as to the conditions necessary for the bringing about of this reaction. The biuret reaction alone is therefore no proof as to the protein nature of a substance—for example, urobilin gives a very similar color reaction—and a protein substance can still retain its protein nature, as by the action of nitrous acid or by a splitting off of ammonia, although it does not give the biuret reaction.

The delicacy of the various reagents differs for the different proteids, and on this account it is impossible to give the degree of delicacy for each reaction for all proteids. Of the precipitation reactions, HELLER'S test (if we eliminate the peptones and certain proteoses) is recommended in the first place for its delicacy, though it is not the most delicate reaction, and because it can be performed so easily. Among the precipitation reactions, that with basic lead acetate (when carefully and exactly executed) and the reactions 6, 7, 8, 9, and 11 are the most delicate. The color reactions 1 to 4 show great delicacy in the order in which they are given.[1]

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[1] Centralbl. f. d. med. Wissensch., 1887.

[2] Journ. of Physiol., 30.

[3] Zeitschr. f. physiol. Chem., 44.

[4] *Ibid.*, 12.

[5] Ber. d. d. chem. Gesellsch., 29 and 30.

No proteid reaction is in itself characteristic, and, therefore, in testing for proteids one reaction is not sufficient, but a number of precipitation and color reactions must be employed.

For the quantitative estimation of coagulable proteids the determination by boiling with acetic acid can be performed with advantage, since, by operating carefully, it gives exact results. Treat the proteid solution with a 1–2 per cent common-salt solution, or if the solution contains large amounts of proteid dilute with the proper quantity of the above salt solution, and then carefully neutralize with acetic acid. Now determine the quantity of acetic acid necessary to completely precipitate the proteids in small measured portions o the neutralized liquid which have previously been heated on the water-bath, so that the filtrate does not respond to HELLER's test. Now warm a larger weighed or measured quantity of the liquid on the water-bath, and add gradually the required quantity of acetic acid, with constant stirring, and continue heating for some time. Filter, wash with water, extract with alcohol and then with ether, dry, weigh, incinerate, and weigh again. With proper work the filtrate should not give HELLER's test. This method serves in most cases, and especially so in cases where other bodies are to be quantitatively estimated in the filtrate.

The precipitation by means of alcohol may also be used in the quantitative estimation of proteids. The liquid is first carefully neutralized, treated with some NaCl if necessary, and then alcohol added until the solution contains 70–80 vol. per cent anhydrous alcohol. The precipitate is collected on a filter after 24 hours, extracted with alcohol and ether, dried, weighed, incinerated, and again weighed. This method is only applicable to liquids which do not contain any other substances, like glycogen, which are insoluble in alcohol.

In both of these methods small quantities of proteid may remain in the filtrates. These traces may be determined as follows: Concentrate the filtrate sufficiently, remove any separated fat by shaking with ether, and then precipitate with tannic acid. Approximately 63 per cent of the tannic-acid precipitate, washed with cold water and then dried, may be considered as proteid.

In many cases good results may be obtained by precipitating all the proteid with tannic acid and determining the nitrogen in the washed precipitate by means of KJELDAHL's method. On multiplying the quantity of nitrogen found by 6.25 we obtain the quantity of proteid.

The removal of proteids from a solution may in most cases be performed by boiling with acetic acid. Small amounts of proteid which remain in the filtrates may be separated by boiling with freshly precipitated lead carbonate or with ferric acetate, as described by HOFMEISTER.[2] If the liquid cannot be boiled, the proteid may be precipitated by the very careful addition of lead acetate, or by the addition of alcohol. If the liquid contains substances which are precipitated by alcohol, such as glycogen, then the proteid may be removed by the alternate addition of potassium-mercuric iodide and hydrochloric acid (see Chapter VIII, on Glycogen Estimation), or also by trichloracetic acid as suggested by OBERMAYER and FRÄNKEL.[1]

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[1] In regard to the precipitation and coloration reactions of proteids with aniline dyes see Heidenhain, Pflüger's Arch., **90**, **96**.

Zeitschr. f. physiol. Chem., **2** and **4**.

In the precipitation of proteid as well as the quantitative estimation by means of heat, it must be borne in mind, as shown by SPIRO,[2] that several nitrogenous substances, such as piperidine, pyridine, urea, etc., disturb the coagulation of the proteids.

Synopsis of the Most Important Properties of the Different Groups of Proteids.

As it is not possible to base the classification of the different proteid groups according to their constitution, we are obliged to make use of their different solubilities and precipitation properties in their general characterization. As there exist no sharp differences between the various groups in this regard it is impossible to draw a sharp line between them.

Albumins. These bodies are soluble in water and are not precipitated by the addition of a little acid or alkali. They are precipitated by the addition of large quantities of mineral acids or metallic salts. Their solution in water coagulates on boiling in the presence of neutral salts, but a weak saline solution does not. If NaCl or $MgSO_4$ is added to saturation to a neutral solution in water at the normal temperature or at 30° C. no precipitate is formed; but if acetic acid is added to this saturated solution the albumins readily separate. When ammonium sulphate is added in substance to saturation to an albumin solution a complete precipitation occurs at the ordinary temperature. Of all the native proteids the albumins are the richest in sulphur, containing from 1.6 per cent to 2.2 per cent.

Globulins. These substances are insoluble in water, but dissolve in dilute neutral salt solutions. The globulins are precipitated unchanged from these solutions by sufficient dilution with water, and on heating they coagulate. The globulins dissolve in water on the addition of very little acid or alkali, and on neutralizing the solvent they precipitate again. The solution in a minimum amount of alkali is precipitated by carbon dioxide, but the precipitate may be redissolved by an excess of the precipitant. The neutral solutions of the globulins containing salts are partly or completely precipitated on saturation with NaCl or $MgSO_4$ in substance at normal temperatures. The globulins are completely precipitated by half-saturating with ammonium sulphate. The globulins contain an average amount of sulphur generally not below 1 per cent.

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[1] Obermayer, Wien. med. Jahrbücher, 1888; Fränkel, Pflüger's Arch., 52 and 55.

[2] Zeitschr. f. physiol. Chem., 30.

According to J. Starke[1] the globulins are not soluble in dilute salt solutions, but form alkali proteid compounds whose solubility in salts is brought about by an increase in the free OH ions produced by the salts. This view is not tenable for several globulins and seems in fact not to be well founded.

That a sharp line cannot be drawn between the albumins and globulins follows from the fact that the albumins can be converted into globulins. The possibility of a conversion of ovalbumin into globulin is based upon the observations of Starke. That a transformation of seralbumin into serglobulin by the aid of the weak action of alkali in the warmth, with the splitting off of sulphur, can take place, has been more conclusively shown by Moll[2] by experimenting with blood-serum as well as with crystalline seralbumin. According to Moll first pseudoglobulin is formed from the seralbumin and then euglobulin (see Chapter VI). The artificial globulins thus obtained had the same sulphur content and properties as the natural products.

It is just as difficult to draw a sharp line between the globulins and albuminates as it is between the globulins and albumins. Several globulins are very readily changed by the action of very little acid, as also by standing under water when in a precipitated condition, into albuminates, and then become insoluble in neutral salt solutions. Osborne,[3] who has closely studied this property in connection with edestin (from hemp-seed), considers the globulin, "globan," which has been made insoluble in salt solution, as an intermediate step in the formation of the albuminate which is produced by the hydrolytic action of the H ions of water or of the acid.

Nucleoalbumins. This group of phosphorized proteids is found widely distributed in both the animal and vegetable kingdoms. The nucleoalbumins behave like weak acids; they are nearly insoluble in water, but dissolve easily with the aid of a little alkali. The nucleoalbumins resemble certain of the globulins and albuminates in solubility and precipitation properties, but differ from these two groups by containing phosphorus. They stand close to the nucleoproteids by their content of phosphorus, but differ from these in not yielding any purine bases on cleavage. It has not yet been found possible to obtain from the nucleoalbumins any proteid-free pseudonucleic acids corresponding to the nucleic acids, but only acids rich in phosphorus, which always give the proteid reactions (Levene and Alsberg, Salkowski[4]). For this reason the nucleoalbumins cannot be classed as compound proteids. In peptic digestion a proteid rich in phosphorus can be split off from most nucleoalbumins, and this has been called *para-* or *pseudonuclein.* The claim made by LIEBERMANN that the pseudonuclein is a combination of proteid with metaphosphoric acid has been shown to be incorrect by the investigations of GIERTZ.[1] The nucleoalbumins always seem to contain some iron.

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[1] Zeitschr. f. Biologie, **40** and **42**. In regard to the various views on this subject see Wolff and Smits, *ibid.*, **41**; Osborne, l. c.; Hammarsten, Ergebnisse der Physiologie, Jahrg. I, Abt. 1.

[2] Hofmeister's Beiträge, **4** and **7**.

[3] Zeitschr. f. physiol. Chem., **33**.

[4] Levene and Alsberg, *ibid.*, **31**; Salkowski, *ibid.*, **32**; Levene, *ibid.*, **32**.

The separation of pseudonuclein in peptic digestion is no doubt characteristic of the nucleoalbumin group, but the non-appearance of the pseudonuclein precipitate does not entirely exclude the presence of a nucleoalbumin. The extent of such a cleavage is dependent upon the intensity of the pepsin digestion, the degree of acidity, and the relationship between the nucleoalbumins and the digestive fluids. The separation of a pseudonuclein may, as shown by SALKOWSKI, not occur even in the digestion of ordinary casein, and WROBLEWSKI did not obtain any pseudonuclein at all in the digestion of the casein from human milk. WIMAN[2] has also shown in the digestion of vegetable nucleoalbumin that the obtainment of considerable pseudonuclein or none is dependent upon the way in which the digestion is performed. The most essential characteristic of this group of proteids is that they contain phosphorus, and that the xanthine bases are absent in their cleavage products.

The nucleoalbumins are often confounded with nucleoproteids and also with phosphorized glucoproteids. From the first class they differ by not yielding any xanthine bodies when boiled with acids, and from the second group by not yielding any reducing substance on the same treatment.

Lecithalbumins. In the preparation of certain protein substances products are often obtained containing lecithin, and this lecithin can only be removed with difficulty or incompletely by a mixture of alcohol and ether. Ovovitellin (Chapter XIII) is such a protein body containing considerable lecithin, and HOPPE-SEYLER considers it a combination of proteid and lecithin. Similar substances occur in fish-eggs. These last lecithalbumins often have the solubilities of the globulins and are readily soluble in dilute salt solutions. The behavior of the nucleoalbumin of the eggs of the perch shows how easily this solubility may be changed. This nucleoalbumin, which contains considerable amounts of lecithin, is readily soluble in dilute NaCl solution, but at ordinary temperatures it is changed by 0.1 per cent HCl nearly instantaneously and without splitting off lecithin, so that it becomes insoluble in dilute salt solutions (HAMMARSTEN). LIEBERMANN[3] has obtained proteids containing lecithin as an insoluble residue on the peptic digestion of the mucous membrane of the stomach, liver, kidneys, lungs, and spleen. He considers them as combinations of proteid and lecithin and calls them *lecithalbumins*. Further investigation of these bodies is desirable.

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[1] Liebermann, Ber. d. deutsch. chem. Gesellsch., 21; Giertz, Zeitschr. f. physiol. Chem., 28.

[2] Salkowski, Pflüger's Arch., 63; Wróblewski, Beiträge zur Kenntnis des Frauenkaseïns, Inaug.-Diss. Bern, 1894; Wiman, Upsala Läkaref. Förh. (N. F.), 2.

[3] Hoppe-Seyler, Med. chem. Untersuch., 1868; also Zeitschr. f. physiol. Chem., 13, 479; Hammarsten, Skand. Arch. f. Physiol., 17; Liebermann, Pflüger's Archiv, 50 and 54.

Alkali and Acid Albuminates. The native proteids are modified by the action of sufficiently strong acids or alkalies. By the action of alkalies all native albuminous bodies are converted, with the elimination of nitrogen, or by the action of stronger alkali, with the extraction of sulphur also, into a new modification, called alkali albuminate, whose specific rotation is increased at the same time. If caustic alkali in substance or in strong solution be allowed to act on a concentrated proteid solution, such as blood-serum or egg-albumin, the alkali albuminate may be obtained as a solid jelly which dissolves in water on heating, and which is called "LIEBERKÜHN's solid alkali albuminate." By the action of dilute caustic alkali solutions on dilute proteid solutions we have alkali albuminates formed slowly at the ordinary temperature, but more rapidly on heating. These solutions may vary with the nature of the proteid acted upon, and also with the intensity of the action of the alkali, but still they have certain reactions in common.

If proteid is dissolved in an excess of concentrated hydrochloric acid, or if we digest a proteid solution acidified with 1–2 p. m. hydrochloric acid in the thermostat, or digest the proteid for a short time with pepsin-hydrochloric acid, we obtain new modifications of proteid which indeed may show somewhat varying properties, but have certain reactions in common. These modifications, which may be obtained in a solid gelatinous condition on sufficient concentration, are called acid albuminates or acid albumins, and sometimes syntonin, though we prefer to apply the term syntonin to the acid albuminate, which is obtained by extracting muscles with hydrochloric acid of 1 p. m.

The alkali and acid albuminates have the following reactions in common: They are nearly insoluble in water and dilute common-salt solution (see page 46), but they dissolve readily in water on the addition of a very small quantity of acid or alkali. Such a solution as nearly neutral as possible does not coagulate on boiling, but is precipitated at the normal temperature on neutralizing the solvent by an alkali or an acid. A solution of an alkali or acid albuminate in acid is easily precipitated on saturating with NaCl, but a solution in alkali is precipitated with difficulty or not at all, according to the amount of alkali it contains. Mineral acids in excess precipitate solutions of acid as well as alkali albuminates. The nearly neutral solutions of these bodies are also precipitated by many metallic salts.

Notwithstanding this agreement in the reactions, the acid and alkali albuminates are essentially different, for by dissolving an alkali albuminate in some acid no acid albuminate solution is obtained, nor is an alkali albuminate formed on dissolving an acid albuminate in water by the aid of a little alkali. In the first case we obtain a combination of the alkali albuminate and the acid soluble in water, and in the other case a soluble combination of the acid albuminate with the alkali added. The chemical process in the modification of proteids with an acid is essentially different from the modification with an alkali, hence the products are of a different kind. The alkali albuminates are relatively strong acids. They may be dissolved in water with the aid of $CaCO_3$, with the elimination of CO_2, which does not occur with typical acid albuminates, and they show in opposition to the acid albuminates also other variations which stand in connection with their strongly marked acid nature. Dilute solutions of alkalies act more energetically on proteids than do acids of corresponding concentration. In the first case a part of the nitrogen, and often also the sulphur, is split off, and from this property we may obtain an alkali albuminate by the action of an alkali upon an acid albuminate; but we cannot obtain an acid albuminate by the obverse reaction (K. MÖRNER[1]). For this reason the designation of the modified proteid obtained by the action of alkali or acid as *protein,* the combination of this protein with alkali as alkali albuminate, and the combination with acid as acid albuminate, leads to a misunderstanding or to a wrong conception.

The preparation of the albuminates has been given above. The corresponding albuminate obtained by the action of alkalies or acids upon a proteid solution may be precipitated by neutralizing with acid or alkali. The washed precipitate is dissolved in water by the aid of a little alkali or acid, and again precipitated by neutralizing the solvent. If this precipitate which has been washed in water is treated with alcohol and ether, the albuminate will be obtained in a pure form.

In the preparation of acid as well as of alkali albuminates, proteoses and the nearly related albuminates are formed. The "*alkali albumose*" obtained by MAAS[2] belongs to this class. The *lysalbinic acid* and *protalbinic acid* obtained by PAAL[3] from ovalbumin are likewise alkali albuminates. *Desaminoalbuminic acid* is an alkali albuminate which SCHMIEDEBERG[4] obtained by the action of such weak alkali that a part of the nitrogen was evolved, but the quantity of sulphur remained the same. The proteid combination obtained by BLUM by the action of formol on proteid and called by him *protogen* has similarities with the alkali albuminates in regard to solubilities and precipitation, but is not identical therewith.[5]

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[1] Pflüger's Arch., 17.

[2] Zeitschr. f. physiol. Chem., **30**.

[3] Ber. d. d. chem. Gesellsch., **35**.

[4] Arch. f. exp. Path. u. Pharm., **39**.

[5] Blum, Zeitschr. f. physiol. Chem., **22**. The older investigations of Loew may be found in Maly's Jahresber., 1888. On the action of formaldehyde see also Benedicenti, Arch. f. (Anat. u.) Physiol., 1897; S. Schwartz, Zeitschr. f. physiol. Chem., **30**; Bliss and Novy, Journ. of Exper. Med., **4**.

Proteoses and Peptones. Peptones were formerly designated as the final products of the decomposition of protein bodies by means of proteolytic enzymes, in so far as these final products are still true proteins, while the intermediate products produced in the peptonization of proteins, in so far as they are not substances similar to albuminates, were designated as proteoses (albumoses, or propeptones). Proteoses and peptones may also be produced by the hydrolytic decomposition of the proteins with acids or alkalies, and by the putrefaction of the same. They may also be formed in very small quantities as by-products in the investigations of animal fluids and tissues, and the question as to the extent to which these exist preformed under physiological conditions requires very careful investigation.

Between the peptone, which represents the final cleavage product, and the proteose, which stands closest to the original protein, we have undoubtedly a series of intermediate products. Under such circumstances it is a difficult problem to try to draw a sharp line between the peptone and the proteose group, and it is just as difficult to define our conception of peptones and proteoses in an exact and satisfactory manner.

The *proteoses* (or *albumoses*) used to be considered as those protein bodies whose neutral or faintly acid solutions do not coagulate on boiling, and which, to distinguish them from peptones, were characterized chiefly by the following properties: The watery solutions are precipitated at the ordinary temperature by nitric acid, as well as by acetic acid and potassium ferrocyanide, and this precipitate has the peculiarity of disappearing on heating and reappearing on cooling. If a proteose solution is saturated with NaCl in substance, the proteose is partly precipitated in neutral solutions, but on the addition of acid saturated with salt it is more completely precipitated. This precipitate, which dissolves on warming, is a combination of the proteose with the acid.

We formerly designated as *peptones* those protein bodies which are readily soluble in water and which do not coagulate by heat, whose solutions are precipitated neither by nitric acid, nor by acetic acid and potassium ferrocyanide, nor by neutral salts and acid.

The reactions and properties which the proteoses and peptones have in common were formerly considered as the following: They give all the color reactions of the proteins, but with the biuret test they give a more beautiful red color than the ordinary proteids. They are precipitated by ammoniacal lead acetate, by mercuric chloride, tannic, phosphotungstic, and phosphomolybdic acids, by potassium-mercuric iodide and hydrochloric acid, and also by picric acid. They are precipitated but not coagulated by alcohol, that is, the precipitate obtained is soluble in water even after being in contact with alcohol for a long time. The proteoses and peptones also have a greater diffusive power than native proteins, and the diffusive power is greater the nearer the questionable substance stands to the final product, the now so-called true peptone.

These old views have gradually undergone an essential change. After Heynsius'[1] observation that ammonium sulphate was a general precipitant for proteins, and for peptones in the old sense, Kühne and his pupils[2] proposed this salt as a means of separating proteoses and peptones. Those products of digestion which separate on saturating their solution with ammonium sulphate, or can indeed be salted out at all, are considered by ~~Kühne and~~ also by most of the modern investigators ~~as~~ proteoses, while those which remain in solution are called peptones or true peptones. These true peptones are formed in relatively large amounts in pancreatic digestion, while in pepsin digestion they are formed only in small quantities or after prolonged digestion.

According to Schützenberger and Kühne[3] the proteins yield two chief groups of new protein bodies when decomposed by dilute mineral acids or with proteolytic enzymes; of these the *anti group* shows a greater resistance to further action of the acid and enzyme than the other, namely, the *hemi group*. These two groups are, according to Kühne, united in the different proteoses, even though in various relative amounts, and each proteose contains the anti as well as the hemi group. The same is true for the peptone obtained in pepsin digestion, hence he calls it *amphopeptone*. In tryptic digestion a cleavage of the amphopeptone takes place into *antipeptone* and *hemipeptone*. Of these two peptones the hemipeptone is further split into amino-acids and other bodies, while the antipeptone is not attacked. By the sufficiently energetic action of trypsin only one peptone is at last obtained, the so-called antipeptone.

Kühne and his pupils, who have conducted extensive investigations on the proteoses and peptones, classify the various proteoses according to their different solubilities and precipitation properties. In the pepsin digestion of fibrin[4] they obtained the following proteoses: (*a*) *Heteroproteose*, insoluble in water but soluble in dilute salt solution; (*b*) *Protoproteose*, soluble in salt solution and water. These two proteoses are precipitated by NaCl in neutral solutions, but not completely. Heteroproteose may, by being in contact with water for a long time or by drying, be converted into a modification, called (*c*) *Dysproteose*, which is insoluble in dilute salt solutions. (*d*) *Deuteroproteose* is a proteose which is soluble in water and dilute salt solution and which is incompletely precipitated from acid solution by saturating with NaCl, and is not precipitated from neutral solutions. This precipitate is a combination of the proteose with acid (HERTH[1]). The deuteroproteose is essentially the same thing that BRÜCKE has designated as peptone.

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[1] Pflüger's Archiv, 34.

[2] See Kühne, Verhandl. d. naturhistor. Vereins zu Heidelberg (N. F.), 3; J. Wenz, Zeitschr. f. Biologie, 22; Kühne and Chittenden, Zeitschr. f. Biologie, 22; R. Neumeister, *ibid.*, 23; Kühne, *ibid.*, 29.

[3] Schützenberger, Bull. de la Soc. chimique de Paris, 23; Kühne, Verhandl. d. naturhist. Vereins zu Heidelberg (N. F.), 1, and Kühne and Chittenden, Zeitschr. f. Biologie, 19. See also Paal, Ber. d. deutsch. chem. Gesellsch., 27.

[4] See Kühne and Chittenden, Zeitschr. f. Biologie, 20.

The proteoses obtained from different protein bodies do not seem to be identical, but differ in their behavior to precipitants. Special names have been given to these various proteoses according to the mother-protein, namely, *albumoses, globuloses, vitelloses, caseoses, myosinoses*, etc. These various proteoses are further distinguished, as *proto-*, *hetero-*, and *deuterocaseoses*, for example. CHITTENDEN[2] has suggested the common name proteoses for the products formed intermediary between the proteins and peptones in the digestion of animal and vegetable proteins. We have made use of it in this sense in preference to the word albumose (which is used in the German and by some other writers), but which will be used in this book as indicating the intermediary products in the hydrolysis of albumins and not as a general term. Certain proteoses have also been obtained in a crystalline state (SCHRÖTTER).

NEUMEISTER[3] designates as *atmidalbumose* that body which is obtained by the action of superheated steam on fibrin. At the same time he also obtained a substance called *atmidalbumin*, which stands between the albuminates and the proteoses.

Of the soluble proteoses NEUMEISTER designates the protoproteose and heteroproteose as *primary proteoses*, while the deuteroproteoses, which are closely allied to the peptones, he calls *secondary proteoses*. As essential differences between the primary and secondary proteoses he suggests the following:[4] The primary proteoses are precipitated by nitric acid in salt-free solutions, while the secondary proteoses are precipitated only in salt solutions, and certain deuteroproteoses, such as deuterovitellose and deuteromyosinose, are precipitated by nitric acid only in solutions saturated with NaCl. The primary proteoses are precipitated from neutral solutions by copper-sulphate solution (2:100), and by NaCl in substance, while the secondary proteoses are not. The primary proteoses are completely precipitated from a solution saturated with NaCl by the addition of acetic acid saturated with salt, while the secondary proteoses are only partly precipitated. The primary proteoses are readily precipitated by acetic acid and potassium ferrocyanide, while the secondary are only incompletely precipitated after some time. The primary proteoses are also, according to PICK,[1] completely precipitated by ammonium sulphate (added to one-half saturation), while the secondary proteoses remain in solution.

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[1] Monatshefte f. Chem., 5.

[2] Kühne and Chittenden, Zeitschr. f. Biologie, **22** and **25**; Neumeister, *ibid.*, **23**; Chittenden and Hartwell, Journ. of Physiol., **11** and **12**; Chittenden and Painter, Studies from the Laboratory, etc., Yale University, **2**, New Haven, 1887; Chittenden. *ibid.*, **3**; Sebelien, Chem. Centralblatt, 1890; Chittenden and Goodwin, Journ. of Physiol., **12**.

[3] Zeitschr. f. Biologie, **26**. See also Chittenden and Meara, Journ. of Physiol., **15**, and Salkowski, Zeitschr. f. Biologie, **34** and **37**.

[4] Neumeister, Zeitschr. f. Biologie, **24** and **26**.

The true peptones, as they were formerly considered to be, are exceedingly hygroscopic, and if perfectly dry, sizzle like phosphoric anhydride when treated with a little water. They are exceedingly soluble in water diffuse more readily than the proteoses, and are not precipitated by ammonium sulphate. In contradistinction to the proteoses, the true peptones are not precipitated by nitric acid (even in solutions saturated with salt), by sodium chloride and acetic acid saturated with salt, potassium ferrocyanide and acetic acid, picric acid, trichloracetic acid, potassium-mercuric iodide, or hydrochloric acid. They are precipitated by phosphotungstic acid, phosphomolybdic acid, corrosive sublimate (in the absence of neutral salts), absolute alcohol, and tannic acid, but the precipitate may redissolve on the addition of an excess of the precipitant. As an important difference between amphopeptone and antipeptone we must also mention that the former gives MILLON's reaction, while the antipeptone does not.

In regard to the precipitation by alcohol we must call attention to the observations of FRÄNKEL that not only are the acid combinations of peptone (PAAL) soluble in alcohol, but also the free peptone, and FRÄNKEL has even suggested a method of preparation based on this behavior. SCHRÖTTER[2] has also prepared crystalline proteoses which were soluble in hot alcohol, especially methyl alcohol.

The views on the hydrolytic cleavage products of peptic and tryptic digestion which were accepted until a few years ago have recently been considerably modified in several points. As this question of peptones is at the present time undergoing active development, and as it is also very complicated and still not clear in many points, it is at present not possible to give a clear, short, and still comprehensive discussion of the subject. We can give here only the most important results.

The older view that in peptic digestion only proteoses and peptones, but no simpler cleavage products, are formed has been shown not to be true. The works of ZUNZ, PFAUNDLER, SALASKIN, LAWROW, LANGSTEIN,[3] and others have shown that simpler products can be produced, some whose nature is still unknown, while others are known, such as alanine, leucine, leucinimide, aminovalerianic acid, aspartic and glutamic acids, phenylalanine, tyrosine, pyrrolidine-carboxylic acid, and lysine and on further cleavage indeed also oxyphenylethylamine, tetra- and pentamethylenediamine. It has not been possible to cause a disappearance of the biuret reaction, and the occurrence of tryptophane is somewhat disputed. MALFATTI obtained tryptophane in peptic digestion only when he used a certain apparently impure preparation of pepsin, and on using pepsin purified according to PEKELHARING it was absent. According to PEKELHARING,[1] purified pepsin also yields tryptophane when the solution is rich in pepsin, and also when the acidity is not too strong, in the presence of small amounts of pepsin.

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[1] Zeitschr. f. physiol. Chem., 24.

[2] Fränkel. Zur Kenntnis der Zerfallsprodukte des Eiweisses bei peptischer und tryptischer Verdauung, Wien, 1896; Schrötter, Monatshefte f. Chem., 14 and 16.

[3] Zunz, Zeitschr. f. physiol. Chem., 28, and Hofmeister's Beiträge, 2; Pfaundler, Zeitschr. f. physiol. Chem., 30; Salaskin, *ibid.*, 32; Salaskin and Kowalewsky, *ibid.*, 38; Lawrow, *ibid.*, 33; Langstein, Hofmeister's Beiträge, 1 and 2.

In connection with the above-mentioned experimental results it must be remarked that not all the products found, for example the oxyphenylethylamine and the diamines, are produced by the action of pepsin, but rather by the action of other enzymes. In certain cases, undoubtedly, impure pepsin was used, or indeed autodigestion of the stomach was carried on, and the action of other enzymes was not excluded. In other cases the digestion with pepsin and considerable acid (even 1 per cent H_2SO_4) was continued for a very long time, indeed for an entire year, without controlling the influence of the acid alone upon the proteoses.

KÜHNE'S view that in tryptic digestion always a peptone, so-called antipeptone, remains which cannot be further split is not strictly true. By sufficiently long autodigestion of the pancreas KUTSCHER[2] was able to obtain as final products a mixture of digestion products which failed to respond to the biuret test. In this connection we must remark that the pure antipeptone (see below), isolated by SIEGFRIED, could be split by trypsin only with great difficulty, and also that the complete disappearance of the biuret reaction in tryptic digestion does not show that a complete decomposition into amino-acids has taken place. According to E. FISCHER and ABDERHALDEN,[3] polypeptide-like bodies are produced, especially in tryptic digestion, and these bodies resist the prolonged action of the enzyme, but yield several different amino-acids on hydrolytic cleavage by acids. The same is probably also true for peptic digestion (see below), and the difference in the digestive products between pepsin and trypsin digestion consists essentially only in that in the first case the cleavage is slower and does not proceed so far, hence the biuret reaction remains and no formation of tryptophane takes place.

By the use of the methods specially worked out by the HOFMEISTER school, of fractionally salting out with ammonium sulphate or zinc sulphate, numerous attempts to separate the various proteoses and peptones have recently been made by UMBER, ALEXANDER, PFAUNDLER, and especially by PICK and ZUNZ.[1] Not only have we learned by these methods of a larger number of proteoses, but our older conception of the products formed primarily has been materially modified. Immediately at the commencement of digestion, even in peptic digestion, a splitting of the protein molecule into several complexes takes place. In opposition to the view of HUPPERT,[2] that the proteoses, in pepsin digestion, are always derived from the primarily formed acid albuminate, PICK and ZUNZ have shown that several proteoses, as well as acid albuminate, appear as primary products at the commencement of the digestion. According to GOLDSCHMIDT[3] a splitting off of proteoses and the formation of acid albuminate takes place simultaneously by the action of dilute acids alone. Besides the proteoses we have, according to ZUNZ and PFAUNDLER, even at the beginning, also other primary bodies, which cannot be salted out and which do not give the biuret reaction, but are in part precipitated by phosphotungstic acid. These little known products seem to be intermediate between the peptones and the amino-acids, and they correspond probably to the polypeptide bodies obtained by FISCHER and ABDERHALDEN in tryptic digestion.

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[1] Malfatti, Zeitschr. f. physiol. Chem., **31**; Pekelharing, Archives d. scienc. biolog. de St. Pétersbourg, **11**; Pawlow Festband.

[2] Zeitschr. f. physiol. Chem., **25**, **26**, **28**, and Die Endprodukte der Trypsinverdauung, Habilitationsschrift Strassburg, 1899.

[3] Zeitschr. f. physiol. Chem., **39**.

By fractional precipitation of WITTE's peptone with ammonium sulphate PICK has obtained various chief fractions of proteoses. The first contains the proto- and heteroproteoses, whose precipitation limit lies at 24–42 per cent saturation with ammonium sulphate solution, i.e., the presence of 24–42 c.c. of the saturated ammonium sulphate solution in 100 c.c. of the liquid. Then follows a fraction A at 54–62 per cent saturation, then a third fraction B, with 70–95 per cent saturation, and finally fraction C, which precipitates from the saturated solution on acidification with sulphuric acid saturated with the salt.

The hetero- and protoproteoses are not, according to our present views, the only primary proteoses. In the proteose fraction B, obtained on saturating with ammonium sulphate in neutral liquids, primary proteoses are also found. As examples we may mention the glucoproteose (PICK) which contains a carbohydrate group, and HOFMEISTER'S[4] synproteose. Unequal responsiveness to the salting-out process is no longer sufficient to differentiate between the primary and secondary proteoses, especially as HASLAM[5] has shown that the products obtained by fractionation are not units but mixtures. According to HASLAM, it is possible to separate the various proteoses by salting out only by following the directions he suggests.

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[1] Umber, Zeitschr. f. physiol. Chem., 25; Alexander, *ibid.*, 25; Pfaundler, *ibid.*, 30; Zunz, *ibid.* 28. and Hofmeister's Beiträge, 2; Pick, *ibid.*, 2, and Zeitschr. f. physiol. Chem., 24 and 28.

[2] Schütz and Huppert, Pflüger's Arch., 80.

[3] F. Goldschmidt, Ueber die Einwirkung von Säuren auf Eiweissstoffe, Inaug.-Diss. Strassburg, 1898.

[4] Ueber Bau und Gruppirung der Eiweisskörper, Ergebnisse der Physiol., Jahrg. I, Abt. 1, 783.

[5] Journ. of Physiol., 32.

Under these circumstances we cannot enter into a discussion of the properties of various proteoses thus far prepared. The differences which exist between the hetero- and protoproteoses obtained from fibrin (PICK[1]) are of great interest. The heteroproteose is insoluble in 32 per cent alcohol, yields only very little tyrosine or indol, but gives abundant leucine and glycocoll, and contains about 39 per cent of the total nitrogen in a basic form. The protoproteose, on the contrary, is soluble in 80 per cent alcohol, yields considerable tyrosine and indol, only little leucine but no glycocoll, and contains only about 25 per cent basic nitrogen. FRIEDMANN, HART, and LEVENE have obtained very similar results in regard to the quantity of basic nitrogen in the two proteoses, although LEVENE did not find the same results as PICK in regard to the amounts of leucine, tyrosine, and glycocoll in the two proteoses. However, HASLAM has shown that PICK'S heteroproteose was contaminated by a proteose which was readily precipitated by alcohol and called α-protoproteose and that there also exists besides this a second protoproteose, β-protoproteose, which is probably identical with PICK'S proteose; still this does not change the fundamental fact that we have a hetero- and a protoproteose which differ essentially in chemical constitution. HART[2] also showed that the heteroproteose (from muscle syntonin) is considerably richer in arginine and poorer in histidine than the protoproteose.

According to PICK, the heteroproteose is also more resistant towards trypsin digestion than the protoproteose, a behavior which coincides with KÜHNE'S view of a resistant atomic complex, an antigroup, in the protein bodies. KÜHNE and CHITTENDEN[3] obtained regularly on the tryptic digestion of heteroproteose a separation of so-called antialbumid, a body which is attacked with great difficulty in tryptic digestion, but which separates as a jelly-like mass and which is richer in carbon (57.5–58.09 per cent), but poorer in nitrogen (12.61–13.94 per cent), than the original protein.

This antialbumid has recently attracted further attention, because, as first found by DANILEWSKY and as other investigators, OKUNEW, SAWJALOW, LAWROW, and SALASKIN and KURAJEFF, have further shown, solutions of rennin, gastric juice, pancreatic juice, and papain cause a coagulum in not too dilute proteose solutions. These coagula, called *plasteines* (coagulum by rennin) by SAWJALOW, and *coaguloses* (coagulum by papain) by KURAJEFF,[4] are similar in many respects to antialbumid, having a higher content of carbon (57–60 per cent) and nitrogen (13–14.6 per cent). They are produced only from proteoses and not from peptones, and form only a small fraction of the related proteose. We cannot state anything with positiveness at present in regard to their importance. It is evident from their composition that they do not represent the reformation of protein from the proteoses, as claimed by some investigators, and their protein nature has indeed been disputed.

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[1] Zeitschr. f. physiol. Chem., **28**.

[2] Friedmann, *ibid.*, **29**; Hart, *ibid.*, **33**; Haslam, l. c.; Levene, Journ. of Biol. Chem., **1**.

[3] Kühne and Chittenden, Zeitschr. f. Biologie, **19**, **20**.

[4] The works of Danilewsky and Okunew are cited and reviewed in the following:

By fractional treatment of Witte's peptone with alcohol and acetone H. Bayer[1] has shown that the substance *plasteinogen*, from which plastein is produced, is not a true protein. This substance was soluble in alcohol-acetone and gave on further purification neither the Millon reaction nor the biuret test. Its composition also differs from the proteins, containing C 38.43, H 7.01, N 8.05 per cent, and the relation of C:N was 4.755:1. According to these investigations plasteinogen is not a proteose but may rather be considered as a peptoid.

It is also generally admitted that the peptones are mostly mixtures of various bodies.[2] Only those peptones isolated by Siegfried and his pupils Mühle, Fr. Müller, Borkel, Krüger, and Scheermesser[3] must be considered as chemical individuals. All these peptones have a pronounced acid character and form salts with carbonates with the evolution of carbon dioxide; they are levorotatory and show a constant degree of rotation. The pepsin-fibrin peptones, α and β, isolated and studied by Siegfried, Mühle, and Borkel, have the formulæ $C_{21}H_{34}N_6O_9$ and $C_{21}H_{36}N_6O_{10}$, respectively. The β-peptone seems to be converted into α-peptone on the splitting off of water. These pepsin peptones give the biuret test as well as Millon's reaction. Their solutions are not precipitated by tannic acid, picric acid, corrosive sublimate, phosphotungstic acid, or alcohol, but are precipitated by basic lead acetate, metaphosphoric acid, and acetic acid and potassium ferrocyanide. The pepsin peptone may be considered as an amphopeptone in Kühne's sense, for in trypsin digestion amino-acids are formed, and all the tyrosine and arginine are split off and antipeptone is formed. The α-pepsin-fibrin peptone is, like the pepsin-glutin peptone, a tribasic acid as well as a biacidic base (Neumann).[4]

The trypsin-fibrin antipeptones studied by Siegfried and Müller have

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Sawjalow, Pflüger's Arch., **85**, and Centralbl. f. Physiol., **16**; Lawrow and Salaskin, Zeitschr. f. physiol. Chem., **36**; Kurajeff, Hofmeister's Beiträge, **1** and **2**; see also Sacharow, Biochem. Centralbl., **1**, 233.

[1] Hofmeister's Beiträge, **4**.

[2] See Kutscher, l. c.; Fränkel and Langstein, Wien. Sitzungsber. Math.-Naturw. Klasse, **110**, 1901; Pick, Hofmeister's Beiträge, **2**.

[3] Siegfried, Arch. f. (Anat. u.) Physiol., 1894; also Zeitschr. f. physiol. Chem., **21**, **43**, and **45**; Siegfried and his pupils, *ibid.*, **38**; Scheermesser, *ibid.*, **41**.

[4] Zeitschr. f. physiol. Chem., **45**.

the formulæ α, $C_{10}H_{17}N_3O_5$, and β, $C_{11}H_{19}N_3O_5$. They have a different specific rotation and are at the same time, according to NEUMANN, bibasic acids and monoacidic bases. The fact that two different antipeptones are formed from the pepsin-fibrin peptone shows that this latter contains at least two antigroups, and not, as KÜHNE claimed, only one. The antipeptones do not give the biuret test, but respond to MILLON'S reaction, and contain no tyrosine groups. They are precipitated by alcohol, but are precipitated less readily or less completely by the reagents which precipitate the pepsin peptones. They have a persistent resistance towards further cleavage by trypsin. On hydrolysis with mineral acids they yield arginine, lysine, glutamic acid, and it seems also aspartic acid. The quantity of basic nitrogen is less than 25 per cent, and the nitrogen split off as ammonia in antipeptone is β 16.1 and α 21.9 per cent of the total nitrogen.

The glutin peptones isolated by SIEGFRIED and KRÜGER have the formulæ $C_{21}H_{39}N_7O_{10}$ for the pepsin-glutin peptone and $C_{19}H_{30}N_6O_9$ for the β-trypsin-glutin peptone. The composition of the apparently very pure pepsin-glutin peptone as prepared by SCHEERMESSER was $C_{21}H_{39}N_7O_{10}$. It gave the biuret test, but did not give the other protein color reactions. Its solution became cloudy with picric acid but was not precipitated. Tannic acid gave a precipitate which was soluble in acetic acid, while phosphotungstic acid produced a precipitate only in concentrated solution. Of the total nitrogen 25 per cent existed as basic and 69.85 per cent as amino-acid nitrogen. It yielded arginine, lysine, glutamic acid, and glycocoll as hydrolytic cleavage products; this peptone contained no histidine.

From glutin peptone, SIEGFRIED, on warming with hydrochloric acid, obtained a base, $C_{21}H_{39}N_9O_8$, which can also be directly obtained from gelatine. This he calls a *kyrin* because it is to be considered as a basic protein nucleus, and he calls this special one *glutokyrin*. The glutokyrin gives the biuret reaction and is considered as a basic peptone. On complete hydrolytic cleavage it yields arginine, lysine, glutamic acid, and glycocoll. Of the total nitrogen two-thirds belongs to the bases and one-third to the amino-acids. Similar basic nuclei, *protokyrins*, have recently been obtained by SIEGFRIED[1] from fibrin and casein, using the same method. Caseinokyrin gives a non-crystalline sulphate but a crystalline phosphotungstate. The free caseinokyrin has an alkaline reaction, gives the biuret test, and its composition corresponds to the formula $C_{23}H_{47}N_9O_8$. It yields arginine, lysine, and glutamic acid on cleavage. The basic nitrogen amounts to about 85 per cent of the total nitrogen, and hence caseinokyrin behaves in this respect like a protamine.

SKRAUP and ZWERGER have presented certain doubts, based upon their own investigations, as to the individuality of the products designated kyrins by SIEGFRIED. SIEGFRIED[1] repudiates the claims made by these investigators, criticises their methods and presents new claims for the individuality of the kyrins, describing exactly the properties of their phosphotungstates and picrates. The constant composition of the sulphate is of importance. It was obtained thus only after repeated recrystallization (reprecipitation nine times of a caseinokyrin sulphate), but it remained unchanged on recrystallization up to fifteen times.

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[1] Kgl. Sächs. Ges. d. Wiss., Math.-Phys. Klasse, 1903, and Zeitschr. f. physiol. Chem., **43**.

Among the known cleavage products of proteins, arginine is the only one which, up to the present, is never absent, and for this reason we designate as proteins only those atomic complexes which contain, besides chained monamino-acids, also arginine, or, more simply, show the above two kinds of imide bindings. Hence caseinokyrin, which yields only arginine, lysine, and glutamic acid, and scombrin (see below), which yields only arginine, α-pyrrolidine-carboxylic acid, and alanine are the simplest known proteins.

Scombrin belongs to the group of substances called protamines, which will be treated of later, and these substances are strongly basic, simple of constitution, give the biuret reaction, and are similar to the proteids. According to KOSSEL[2] we can conceive of the formation of the protamines by a successive cleavage of the typical proteins, and the occurrence of basic protokyrins in the hydrolytic cleavage of genuine proteins like gelatine has given valuable support to KOSSEL'S theory as to a basic nucleus in the protein bodies. We must not infer from this that each protein contains only one nucleus. It is, on the contrary, possible and not improbable that each protein is composed of several larger complexes and that each of these contains a special nucleus. The proteoses may be considered as large complexes of this kind which, at least in part, do not separate but seem to stand together. The cleavage of proteins, according to SCHÜTZENBERGER and KÜHNE, into a hemi- and an antigroup, of which the first contains, among other complexes, the readily split tyrosine and tryptophane, while the antigroup contains α-proline (α-pyrrolidine-carboxylic acid), glycocoll, and phenylalanine; the different behaviors of proto- and heteroproteoses; and the occurrence of non-biuret-giving polypeptides in digestion, coincide well with such a view.

On account of the cleavage taking place in digestion, the digestive products should have a lower molecular weight than the original protein. This is really the case. The molecular weight of the different proteins has not been determined with certainty,[3] but it is generally considered as about

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[1] Skraup and Zwerger, Monatshefte f. Chemie, 26; Siegfried, Zeitschr. f. Physiol., 48.

[2] Zeitschr. f. physiol. Chem., 44.

[3] See especially F. N. Schulz, Die Grösse des Eiweissmoleküls, Jena, 1903.

5000–10 000 for the albumins and for casein. The molecular weight for protoproteoses was found by SABANEJEW to be 2467–2643, and 3200 for the deuteroproteoses. The peptones have a still lower molecular weight, being between 400 and 250 for the different peptones (SABANEJEW, PAAL, SJÖQVIST[1]).

The elementary analyses[2] have not given us much information as to the characteristic differences between the various proteoses and most so-called peptones. Certain proteoses, especially those that can be salted out with difficulty, and the peptones differ very materially in composition from the mother-substances and often have a lower carbon content.

Besides the behavior in the salting-out process, attempts have been made to find other points of difference between the peptones and proteoses. SCHRÖTTER and FRÄNKEL[3] consider the sulphur content as a pronounced point of difference. The peptones, according to them, are free from sulphur, while the proteoses, on the contrary, contain sulphur. FRÄNKEL has been able to find only one proteose (in KÜHNE's sense) which did not contain sulphur.

In the preparation and separation of various proteoses and peptones all precipitable protein is always removed first by neutralization and then by boiling. The proteoses may then be separated from the peptones by means of ammonium sulphate according to KÜHNE's method, and divided into different fractions according to the method of PICK and the HOFMEISTER school. The separation and preparation of pure hetero- and protoproteoses can be best performed by the method suggested by PICK, but this method, as well as that with ammonium sulphate, gives good results only when the precautions suggested by HASLAM[4] are carefully followed. As most of the older methods do not give pure substances but rather mixtures, it is perhaps sufficient simply to call attention here to other methods, such as those suggested by K. BAUMANN and BÖMER, P. MÜLLER, FRÄNKEL, SCHRÖTTER, and PAAL. The only method which seems thus far to have led to a pure preparation of peptone is the iron method used by SIEGFRIED.[5]

For the detection of proteoses and peptones in animal fluids we proceed as follows, according to DEVOTO: The coagulable proteins are removed by prolonged heating, and the solution is then saturated with ammonium sulphate. True peptones (besides deuteroproteose not precipitated) may be detected in the cold filtrate by means of the biuret test. The proteoses are contained in the mixture of precipitate and salt crystals collected on the filter. The proteoses are dissolved from this mixture by washing with water, and may be detected in the wash-water by means of the biuret test. According to HALLIBURTON and COLLS[1] traces of proteoses may be formed from other proteins in this method by prolonged heating. As the best method they suggest either the precipitation of the native proteids by the addition of a 10 per cent trichloracetic-acid solution, or the conversion of the native proteins to the insoluble form by the continued action of alcohol. The last method is hardly applicable to blood-serum, as the so-called fibrin-ferment, which also gives the biuret test, is not made insoluble by this procedure.

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[1] Sabanejew, Ber. d. d. chem. Gesellsch., 26, 385; Paal, ibid., 25, 1827; Sjöqvist, Skand. Arch. f. Physiol., 5.

[2] Elementary analyses of proteoses and peptones will be found in the works of Kühne and Chittenden and their pupils cited in foot-note 2, p. 52; also by Herth, Zeitschr. f. physiol. Chem., 1, and Monatshefte f. Chem., 5; Maly, Pflüger's Arch., 9, 20; Henninger, Compt. rend., 86; Schrötter, l. c.; Paal, l. c.

[3] Schrötter, Monatshefte f. Chem., 14 and 16; Fränkel, Zur Kenntnis der Zerfallsprodukte des Eiweiss bei peptischer und tryptischer Verdauung, Wien, 1896.

[4] Kühne, Zeitschr. f. Biologie, 29; Pick, l. c.; Haslam, l. c.

[5] Baumann and Bömer, Chem. Centralbl., 1898, 1, 640; Müller, Zeitschr. f. physiol. Chem., 26; Fränkel, l. c., Zur Kenntnis etc.; Schrötter, Monatshefte f. Chem., 14 and 16; Paal, l. c.; Siegfried, l. c.

If a solution saturated with ammonium sulphate is to be tested for the biuret reaction, it must first be treated with a slight excess of concentrated caustic-soda solution, the solution being kept cold, and after the sodium sulphate has settled, the liquid is treated with a 2 per cent solution of copper sulphate, drop by drop.

The estimation of nitrogen, the biuret test (colorimetric), and the polariscopic method have been used in the quantitative estimation of proteoses and peptones. These two last-mentioned methods do not yield exact results.

Coagulated Proteins. Proteins may be converted into the coagulated condition by different means: by heating, by the action of alcohol, especially in the presence of neutral salts, by chloroform, ether, and metallic salts, and by the prolonged shaking of their solutions (RAMSDEN[2]), and in certain cases, as in the conversion of fibrinogen into fibrin (Chapter VI), by the action of an enzyme. The nature of the processes which take place during coagulation is unknown. The coagulated albuminous bodies are insoluble in water, in neutral salt solutions, and in dilute acids or alkalies, at normal temperature. They are dissolved and converted into albuminates by the action of less dilute acids or alkalies, especially on heating.

Coagulated proteins also seem to occur in animal tissues. We find, at least in many organs such as the liver and other glands, proteins which are not soluble in water, dilute salt solutions, or very dilute alkalies, and only dissolve after being modified by strong alkalies.

Histones are basic proteins which stand to a certain extent between the strongly basic protamines (see below) and the true proteins. Their content of nitrogen varies between 16.5 and 19.8 per cent and in certain instances is not higher than in other proteins, especially vegetable proteins. According to KOSSEL and KUTSCHER and LAWROW they are, on the contrary, richer in basic nitrogen and especially yield more arginine than other proteins. KOSSEL first isolated a peculiar protein substance from the red corpuscles of goose blood which was precipitated by ammonia, and because of its similarity in certain regards to the peptones (in the old sense) he called it histone. At the present time a number of very different bodies are described as histones, such as those obtained from nucleohistone (LILIENFELD), from hæmoglobin (globin according to SCHULZ), from mackerel spermatozoa (scombron according to BANG), from the codfish (gadushistone according to KOSSEL and KUTSCHER), from the burbot, (lotahistone, EHRSTRÖM), and from the sea-urchin (arbacin, MATHEWS).[1]

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[1] Devoto, Zeitschr. f. physiol. Chem., 15; Halliburton and Colls, Journ. of Path. and Bact., 1895.

[2] Arch. f. (Anat. u.) Physiol., 1894.

Sulphur has been found in those histones in which it has been tested for. They give the biuret test, but as a rule only a faint MILLON's reaction. The goose-blood histone first studied by KOSSEL gives the three following reactions: The neutral salt-free solution first, does not coagulate on boiling; second, gives a precipitate with ammonia which is insoluble in an excess of the precipitant; third, gives a precipitate with nitric acid which disappears on heating and reappears on cooling.

The different histones behave differently in these three reactions, and hence they are not specific. On the other hand, all histones seem to be precipitated from neutral solution by alkaloid reagents, and they also produce precipitates in protein solutions. These two reactions are likewise not specific for the histones, as the protamines have a similar behavior. The histones differ from the protamines by having a much lower content of basic nitrogen, and also probably by always containing sulphur. True proteins, as OSBORNE's[2] edestan, also give these two reactions; therefore it is impossible by qualitative tests alone to identify a substance as a histone with positiveness. The large content of basic nitrogen and of arginine is not a sure point of difference between histones and other bodies. Histone yields little more than 40 per cent basic nitrogen, while a heteroproteose yields about the same, namely, 39 per cent. Histone yields 14–15.5 per cent arginine (gadushistone), and the lotahistone only 12 per cent. The plant-globulin edestin[3] yields a much larger amount of arginine, namely, 14.07 per cent. On hydrolytic cleavage the histones, like other proteins, but unlike the protamines, yield a large number of monamino-acids. ABDERHALDEN and RONA[4] obtained from thymus histone the following: leucine 11.8, alanine 3.46, glycocoll 0.50, α-proline 1.46, phenylalanine 2.20, tyrosine 5.20, and glutamic acid 0.53 per cent. According to KOSSEL the histones are probably intermediate bodies between the protamines and protein bodies on the demolition of the latter, and if this is true, then it is not to be expected that histones should have perfectly specific reactions, and for this reason it is hardly possible for the present to give a precise definition for the histones.

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[1] Kossel, Zeitschr. f. physiol. Chem., 8, and Sitzungsber. der Gesellsch. zur Beförd. d. ges. Naturwiss. zu Marburg, 1897; Kossel and Kutscher, *ibid.*, 1900, and Zeitschr. f. physiol. Chem., **31**; Lawrow, *ibid.*, **28**, and Ber. d. d. chem. Gesellsch., **34**; Lilienfeld, Zeitschr. f. physiol. Chem., **18**; Schulz, *ibid.*, **24**; Bang, *ibid.*, **27**; Ehrström, *ibid.*, **32**; Mathews, *ibid.*, **28**.

[2] Zeitschr. f. physiol. Chem., **33**.

[3] See Kossel, Ber. d. d. chem. Gesellsch., **34**, 3236.

[4] Zeitschr. f. physiol. Chem., **41**.

The **parahistone** found by FLEROFF in the thymus gland yields so little basic nitrogen that it probably does not belong to the histone group (KOSSEL and KUTSCHER[1]).

Protamines. In close relationship to the proteins stands a group of substances, the protamines, discovered by MIESCHER, which are designated by KOSSEL as the simplest proteins or as the nucleus of the protein bodies. Thus far they have been found only in combination with nucleic acids in fish spermatozoa. They differ essentially from the proteins by the fact that they yield chiefly diamino-acids (always abundant arginine) as cleavage products, and only a small amount of monamino-acids. They are strongly basic substances rich in nitrogen (about 30 per cent or more) and have high molecular weight.

Protamine was discovered by MIESCHER[2] in salmon spermatozoa. Later KOSSEL isolated and studied similar bases from the spermatozoa of herring, sturgeon, mackerel, and other fishes. As all these bases are not identical, KOSSEL uses the name protamines to designate the group and calls the individual protamines according to their origin *salmine, clupeine, scombrine, sturine, cyprinine, cyclopterine,* etc.

The percentage composition of these bodies has not been satisfactorily determined. As probable formulæ we have for salmine $C_{32}H_{56}N_{18}O_4$ (MIESCHER-SCHMIEDEBERG) or $C_{30}H_{57}N_{17}O_6$ (KOSSEL and GOTO), for clupeine $C_{30}H_{62}N_{14}O_9$, and for sturine $C_{36}H_{69}N_{19}O_7$ (KOSSEL) or $C_{34}H_{71}N_{17}O_9$ (GOTO). On boiling with dilute mineral acids, as also by tryptic digestion, the protamines first yield peptone-like substances called *protones*, from which simpler products are derived on further cleavage. All protamines yield arginine, the four protamines salmine, clupeine, cyclopterine, and sturine yielding 87.4, 82.2, 62.5, and 58.2 per cent respectively. Sturine yields besides this the two hexone bases lysine, 12 per cent, and histidine, 12.9 per cent. Histidine has not been found in any other protamine. The carp protamine, cyprinine, occurs in two different modifications, namely, α- and β-cyprinine. The α-cyprinine yields only little arginine, 4.9 per cent, but the lysine content is pronounced, 28.8 per cent. Of the total nitrogen 30.3 per cent exists as lysine. Kossel and Dakin have obtained from salmine the following cleavage products, namely, arginine 87.4, serine 7.8, aminovalerianic acid 4.3, and α-pyrrolidine-carboxylic acid 11 per cent, and according to them the salmine contains about 10 mol. arginine, 2 mol. serine, 1 mol. aminovalerianic acid, and 2 mol. α-proline. Scombrine contains only arginine, alanine, and α-proline. The following summary according to Kossel[1] gives a view of the cleavage products of the protamines thus far investigated.

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[1] Fleroff, Zeitschr. f. physiol. Chem., 28; Kossel and Kutscher, l. c.

[2] In regard to protamines, see Miescher, Histochemische und Physiologische Arbeiten, Leipzig, 1897; Piccard, Ber. d. deutsch. chem. Gesellsch., 7; Schmiedeberg, Arch. f. exp. Path. u. Pharm., 37; Kossel, Zeitschr. f. physiol. Chem., 22 (Ueber die basischen Stoffe des Zellkerns), 25, 165 and 190, 26, 40, and 44, and Sitzungsber. der Gesellsch. zur Beförd. der ges. Naturwiss. zu Marburg, 1897; Berl. klin. Wochenschr., 1904; Kossel and Mathews, Zeitschr. f. physiol. Chem., 23 and 25; Kossel and Kutscher, ibid., 31; Goto, *ibid.*, 37; Kurajeff, *ibid.*, 32; Morkowin, *ibid.*, 28; Kossel and Dakin, *ibid.*, 40, 41, and 44.

	Scombrine.	Salmine.	Clupeine.	Sturine.	Cyclopterine.	α-Cyprinine.	β-Cyprinine.
Alanine	+	0	+	+	?	?	?
Serine	0	+	+	0	?	?	?
Aminovalerianic acid	0	+	+	0	?	+	+
Leucine	0	0	0	+	?	?	?
Arginine	+	+	+	+	+	+	+
Lysine	0	0	0	+	0	+	+
Histidine	0	0	0	+	0	0	0
α-Proline	+	+	+	0	?	?	?
Tyrosine	0	0	0	0	+	0	0
Tryptophane	0	0	0	0	+	0	0

Solutions of these bases in water are alkaline and have the property of giving precipitates with ammoniacal solutions of proteins or primary proteoses. These precipitates are considered as histones by Kossel. The salts with mineral acids are soluble in water, but insoluble in alcohol and ether. They are more or less readily precipitated by neutral salts (NaCl). Among the salts of the protamines, the sulphate, picrate, and the double-platinum chloride are the most important and are used in the preparation of the protamines. The protamines are, like the proteins, levogyrate. They give the biuret test beautifully, but with the exception of cyclopterine and β-cyprinine do not give Millon's reaction. The protamine salts are precipitated in neutral or even faintly alkaline solutions by phosphotungstic acid, picric acid, chromic acid, and alkali ferrocyanides.

The protamines are prepared, according to Kossel, by extracting the heads of the spermatozoa, which have previously been extracted with alcohol and ether, with dilute sulphuric acid (1–2 per cent), filtering, and precipitating with 4 vols. of alcohol. The sulphate may be purified by repeated solution in water and precipitation with alcohol, and if necessary, conversion into the picrate. For more details see the works of Kossel. The double-platinum salt is best suited for analysis and can be obtained, according to Goto, by precipitating the methyl-alcohol solution of the protamine hydrochloride with platinum chloride. Miescher also precipitates the base as a double-platinum salt.

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[1] Zeitschr. f. physiol. Chem., 44.

II. Compound Proteids.

With this name we designate a class of bodies which are more complex than the proteids, and which yield as primary splitting products proteids on the one side and non-proteid bodies, such as pigments, carbohydrates, nucleic acids, etc., on the other.[1]

The compound proteids known at the present time are divided into three chief groups. These are the *hæmoglobins*, the *glucoproteids*, and the *nucleoproteids*. The hæmoglobins will be discussed in a following chapter (Chapter VI, on the blood).

Glucoproteids are those compound proteids which on decomposition yield a proteid on the one side, and a carbohydrate or derivatives of the same on the other, but no purine bodies. Some glucoproteids are free from phosphorus (mucin substances, chondroproteids, and hyalogens), and some contain phosphorus (phosphoglucoproteids).

The glucoproteids free from phosphorus may, as regards the nature of the carbohydrate groups split off, be divided into two chief groups, the *mucin substances* and the *chondroproteids*. The first yield on hydrolytic cleavage an amino-sugar, which has been shown to be glucosamine in all cases except one.[2] In the chondroproteids, on the contrary, the proteid is united to chondroitin-sulphuric acid.

Mucin Substances. These bodies contain carbon, hydrogen, nitrogen, sulphur, and oxygen. Compared with proteids they are poorer in nitrogen and as a rule have also considerably less carbon. The carbohydrate complex, whose nature has been shown by the investigations of FR. MÜLLER[3] and his pupils, occurs, as it seems, in the mucin substances as a polysaccharide related to chitosan, which on hydrolytic cleavage yields glucosamine (chitosamine), and, at least in most cases, also acetic acid. The mucin substances differ very markedly among one another, hence we divide them into two groups, the mucins and the mucoids.

The *true mucins* are characterized by the fact that their natural solutions, or solutions prepared by the aid of a trace of alkali, are mucilaginous, ropy, and give a precipitate with acetic acid which is insoluble in excess of acid or soluble only with great difficulty. The *mucoids* do not show these physical properties and have other solubilities and precipitation properties. As we have intermediate steps between different protein bodies, so also we have such between true mucins and mucoids, and a sharp line cannot be drawn between these two groups.

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[1] Hoppe-Seyler has given the name *proteide* to these compound proteids, but as this term is misleading in English we do not use it in English classifications in this sense.

[2] See Schulz and Ditthorn, Zeitschr. f. physiol. Chem., 29. When both carbohydrate groups are simultaneously combined with one body, then probably we are not dealing with a chemical individual, but rather with a mixture.

[3] See Fr. Müller, Zeitschr. f. Biologie, 42, which contains all the pertinent literature, and also L. Langstein, Die Bildung von Kohlenhydraten aus Eiweiss, Ergebnisse der Physiologie, Jahrg. I, Abt. 1.

It is just as difficult at present to draw a sharp line between the proteids and the mucins or mucoids, since we have been able to split off carbohydrate complexes from several proteids, and the proteids of the white of egg are undoubtedly glucoproteids. It is immaterial whether we consider these glucoproteids as belonging to the mucoids or to a special group. From a comparative chemical standpoint, they undoubtedly belong to the mucoid group, representatives of which occur in eggs to a considerable extent.

True mucins are secreted by the larger mucous glands, by certain mucous membranes, and by the skin of snails and other animals. True mucin also occurs in the navel-cord. Sometimes, as in snails and in the membrane of the frog-egg (GIACOSA [1]), a mother-substance of mucin, a mucinogen, has been found which may be converted into mucin by alkalies. Mucoid substances are found in certain cysts, in the cornea, the crystalline lens, white of egg, and in certain ascitic fluids. The so-called tendon-mucin, which, according to the recent investigations of LEVENE and of CUTTER, and GIES,[2] contains chondroitin-sulphuric acid or a related substance, cannot be classified as a mucin, but must, like the chondromucoid and the osseomucoid, be classified as chondroproteid. As the mucin question has not been sufficiently studied, it is at the present time impossible to give any positive statements in regard to the occurrence of mucins and mucoids, especially as without doubt in many cases non-mucinous substances have been described as mucins.

1. **True Mucins.** Thus far we have been able to obtain only a few mucins in a pure and unchanged condition, because of the reagents used. The elementary analyses of these mucins have given the following results:

	C	H	N	S	
Mucin from mucous membrane (air-passages)	48.26	6.91	10.7	1.4	(FR. MÜLLER)
Mucin from submaxillary	48.84	6.80	12.32	0.84	(HAMMARSTEN[3])
Mucin from snail	50.32	6.84	13.65	1.75	(HAMMARSTEN[3])
Synovial mucin	51.05	6.53	13.01	1.34	(v. HOLST [4])

MÜLLER obtained 35 per cent glucosamine from mucous-membrane mucin and 23.5 per cent from the submaxillary mucin.

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[1] Giacosa, Zeitschr f. physiol. Chem.. 7; Hammarsten, Pflüger's Archiv, **36**, and Skand. Arch. f. Physiol., **17**.

[2] Levene, Zeitschr. f. physiol. Chem., **31**; Cutter and Gies, Amer. Journ. of Physiol., **6**.

[3] Fr. Müller, Zeitschr. f. Biologie, **42**; Hammarsten, Zeitschr. f. physiol. Chem., **12**, and Pflüger's Arch., **36**.

[4] Zeitschr. f. physiol Chem., **43**.

By the action of superheated steam on mucin a carbohydrate, animal gum (Landwehr), is split off. This has not been substantiated by other investigators, such as Folin and Fr. Müller.[1] Instead of a non-nitrogenous gum a nitrogenous carbohydrate derivative was always obtained.

On boiling mucin with dilute mineral acids, acid albuminate and bodies similar to proteoses are obtained, besides a reducing substance which is not free glucosamine (Steudel[2]). By the action of strong acids upon mucins or mucoids Otori[3] obtained several of the cleavage products of the proteins, such as leucine, tyrosine, glycocoll, glutamic acid, oxalic acid, guanidine, arginine, lysine, and humus substances, and also carbohydrate cleavage products, such as levulinic acid. Certain mucins, as the submaxillary mucin, are easily changed by very dilute alkalies, as lime-water, while others, such as tendon-mucin, are not affected. If a strong caustic-alkali solution, such as 5 per cent KOH solution, is allowed to act on submaxillary mucin, we obtain alkali albuminate, bodies similar to proteoses and peptones and one or more substances of an acid reaction and with strong reducing powers.

On peptic digestion proteoses and peptone-like bodies, still containing the carbohydrate group, are produced. On tryptic digestion still simpler cleavage products are formed, namely, leucine, tyrosine, and tryptophane (Posner and Gies[4]). The glucosamine, so far as we know, is not split off by proteolytic enzymes, but only after strong hydrolysis with acids, and this speaks against the assumption that the glucosamine group exists as a glucoside-like combination in the mucin molecule (Neuberg and Milchner[5]).

In one or another respect the various mucins act somewhat dissimilarly. For example, the snail and sputum mucins are insoluble in dilute hydrochloric acid of 1-2 p. m., while the mucin of the submaxillary gland and the navel-cord is soluble. The former become flaky with acetic acid, while the submaxillary mucin is precipitated in more or less fibrous, tough masses. Still all the mucins have certain reactions in common.

In the dry state mucin forms a white or yellowish-gray powder. When moist it forms, on the contrary, flakes or yellowish-white tough lumps or masses. The mucins are acid in reaction. They give the color reactions of the proteins. They are not soluble in water, but may give a neutral solution with water with the aid of small amounts of alkali. Such a solution does not coagulate on boiling, but acetic acid gives at the normal temperature a precipitate which is nearly insoluble in an excess of the precipitant. If 5–10 per cent NaCl be added to a mucin solution, this can now be carefully acidified with acetic acid without giving a precipitate. Such acidified solutions are copiously precipitated by tannic acid; with potassium ferrocyanide they give no precipitate, but on sufficient concentration they become thick or viscous. A neutral solution of alkali mucin is precipitated by alcohol in the presence of neutral salts; it is also precipitated by several metallic salts. If mucin is heated on the water-bath with dilute hydrochloric acid of about 2 per cent, the liquid gradually becomes a yellowish or dark brown and reduces copper salts in alkaline solutions.

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[1] Landwehr. Zeitschr. f. physiol. Chem., 8, 9; also Pflüger's Arch., 39 and 40; Folin, Zeitschr. f. physiol. Chem., 23; Fr. Müller, Sitzungsber. d. Gesellsch. zur Beförd. d. gesammt. Naturwiss. zu Marburg, 1896.

[2] Zeitschr. f. physiol. Chem., 34.

[3] *Ibid.*, 42 and 43.

[4] Amer. Journ. of Physiol., 11.

[5] Berl. klin. Wochenschr., 1904.

The mucin most readily obtained in large quantities is the submaxillary mucin, which may be prepared in the following way: The filtered watery extract of the gland, free from form-elements and as colorless as possible, is treated with 25 per cent hydrochloric acid, so that the liquid contains 1.5 p. m. HCl. On the addition of the acid the mucin is immediately precipitated, but dissolves on stirring. If this acid liquid is immediately diluted with 2–3 vols. of water, the mucin separates and may be purified by redissolving in 1–5 p. m. acid, and diluting with water and washing therewith. The mucin of the navel-cord may be prepared in the same way. As a rule the mucins can be prepared by precipitation with acetic acid and repeated solution in dilute lime-water or alkali and reprecipitation with acetic acid. Finally they are treated with alcohol and ether. In the preparation of sputum mucin a very complicated method is necessary (FR. MÜLLER).

The precipitation by acetic acid, as shown by HAMMARSTEN,[1] is not applicable in the preparation of submaxillary mucin, because another proteid substance is precipitated with the mucin, but remains in solution on using the hydrochloric-acid method above described. POSNER and GIES [2] have by special experiments shown the power of mucins of precipitating proteids, and this makes the ordinary method of precipitating with acetic acid questionable.

2. **Mucoids** or **Mucinoids.** In this group we must include those non-phosphorized glucoproteids which are neither true mucins nor chondroproteids, even though they show amongst themselves such differences in behavior that they can be divided into several subgroups of mucoids. To the mucoids belong *pseudomucin*, the probably related body *colloid*, *ovomucoid*, and other bodies, which on account of their differences will be best treated individually in their respective chapters.

Hyalogens. Under this name KRUKENBERG [3] has designated a number of differing bodies, which are characterized by the following: By the action of alkalies they change, with the splitting off of sulphur and some nitrogen, into soluble nitrogenized products called by him *hyalines* and which yield a pure car-

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[1] Zeitschr. f. physiol. Chem., **12**.

[2] Amer. Journ. of Physiol., **11**.

[3] Verh. d. physik.-med. Gesellsch. zu Würzburg, 1883; also Zeitschr. f. Biologie, **22**.

bohydrate by further decomposition. We find that very heterogeneous substances are included in this group. Certain of these hyalogens seem undoubtedly to be glucoproteids. *Neossin*[1] of the Chinese edible swallow's-nest, *membranin*[2] of DESCEMET's membrane and of the capsule of the crystalline lens, and *spirographin*[3] of the skeletal tissue of the worm Spirographis seem to act as such. Others on the contrary, such as *hyalin*[4] of the walls of hydatid cysts, and *onuphin*[5] from the tubes of Onuphis tubicola, do not seem to be compound proteids. The so-called *mucin of the holothures*[6] and *chondrosin*[7] of the sponge, Chondrosia reniformis, and others may also be classed with the hyalogens. As the various bodies designated by KRUKENBERG as hyalogens are very dissimilar, it is not of much advantage to arrange these in special groups.

3. **Chondroproteids** are those glucoproteids which as primary cleavage products yield proteid and an ethereal sulphuric acid containing a carbohydrate, *chondroitin-sulphuric acid*. *Chondromucoid*, occurring in cartilage, is the best example of this group. *Amyloid* occurring under pathological conditions also belongs to this group. On account of the property of chondroitin-sulphuric acid of precipitating proteid, it is also possible that under certain circumstances combinations of this acid with proteid may be precipitated from the urine and be considered as chondroproteids.

The chondromucoid, the so-called tendon-mucin, and the osseomucoid have greatest interest as constituents of cartilage, of the connective tissues, and of the bones, and on this account these bodies and their cleavage product, chondroitin-sulphuric acid, will be treated in a following chapter (X). On the contrary, amyloid, which has always been considered in connection with the protein substances, will be described here.

Amyloid, so called by VIRCHOW, is a protein substance appearing under pathological conditions in the internal organs, such as the spleen, liver, and kidneys, as infiltrations; and in serous membranes as granules with concentric layers. It probably also occurs as a constituent of certain prostate calculi. The chondroproteid occurring under physiological conditions in the walls of the arteries is perhaps, according to KRAWKOW, very nearly related to the amyloid substance, but not identical with it, as shown by NEUBERG.[8]

The amyloid prepared by KRAWKOW and NEUBERG had about the same composition: C 49.0–50.1; H 7–7.2; N 14–14.1, and S 1.8–2.8 per cent. The aorta amyloid of man and of the horse contained respectively C 49.6

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[1] Krukenberg, Zeitschr. f. Biologie, **22**.

[2] C. Th. Mörner, Zeitschr. f. physiol. Chem., 18.

[3] Krukenberg, Würzburg, Verhandl. 1883; also Zeitschr. f. Biologie, 22.

[4] A. Lücke, Virchow's Arch., **19**; also Krukenberg, Vergleichende physiol. Stud., Series 1 and 2, 1881.

[5] Schmiedeberg, Mitth. aus d. zool. Stat. zu Neapel, **3**, 1882.

[6] Hilger, Pflüger's Archiv, **3**.

[7] Krukenberg, Zeitschr. f. Biologie, **22**.

[8] Krawkow, Arch. f. exp. Path. u. Pharm., **40**, which contains the literature; Neuberg, Verhandl. d. d. Pathol. Gesellsch. 1904.

and 50.5; H 7.2; N 14.4 and 13.8; S 2.3 and 2.5 per cent. According to Neuberg, aorta amyloid differs from spleen and liver amyloid by a different division of the nitrogen, which is evident from the following:

	Monamino-N	Diamino-N	Amide-N
Liver amyloid........	43.2	51.2	4.9
Spleen amyloid........	30.6	57.0	11.2
Aorta amyloid........	54.9	36.0	8.8

From liver amyloid Neuberg obtained glycocoll 0.8; leucine 22.2; glutamic acid 3.8; tyrosine 4.0; α-proline 3.1; arginine 13.0, and lysine 11.6 per cent.

By the action of alkali, amyloid splits into protein and chondroitin-sulphuric acid (see Chapter X), and according to Krawkow it is therefore a firm, perhaps ester-like combination of this acid with protein. The protein, from the investigations of Neuberg, is of a basic nature and most comparable to the histones. According to Neuberg, amyloid is a transformation product of the proteins, just as are the protamines, and the differences between liver, spleen, and aorta amyloid indicate various phases of this transformation.

Amyloid is an amorphous white substance, insoluble in water, alcohol, ether, dilute hydrochloric and acetic acids. It is soluble in concentrated hydrochloric acid or caustic alkali with decomposition. On boiling with dilute hydrochloric acid it yields sulphuric acid and a reducing substance. It is not dissolved by gastric juice, according to Krawkow and in agreement with most of the older statements. It is nevertheless changed so that it is soluble in dilute ammonia, while the typical amyloid is insoluble therein. Neuberg finds on the contrary that amyloid (from liver) is digested by pepsin as well as by trypsin, although more slowly than fibrin, and that it is also destroyed in autolysis, so that in life an absorption is possible. Amyloid gives the xanthoproteic reaction and the reactions of Millon and Adamkiewicz. Its most important property is its behavior with certain coloring matters. It is colored reddish brown or a dingy violet by iodine; a violet or blue by iodine and sulphuric acid; red by methylaniline iodide, especially on the addition of acetic acid; and red also by aniline green. Of these color reactions those with aniline dyes are the most important. The iodine reaction appears less constant and is greatly dependent upon the physical condition of the amyloid. The color reactions are due to the presence of the chondroitin-sulphuric acid component.

The preparation of amyloid may be performed as follows according to Modrzejewski and Krawkow.[1] The finely divided organ is exhausted first with water and then with dilute ammonia, which leaves the insoluble amyloid and removes the free or the combined chondroitin-sulphuric acid, besides other substances. The product, after being washed with water, is digested with pepsin for several days at 38° C. The residue, after washing with hydrochloric acid and water, is dissolved in dilute ammonia, filtered, again precipitated with dilute hydrochloric acid, dissolved, if necessary, in ammonia, precipitated a second time with hydrochloric acid, washed with water, the precipitate dissolved in baryta-water, which leaves the nucleins undissolved, and the barium filtrate precipitated with hydrochloric acid, and then washed with water, alcohol, and ether.

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[1] Modrzejewski, Arch. f. exp. Path. u. Pharm., 1; Krawkow, l. c.

Phosphoglucoproteids. This group includes the phosphorized glucoproteids. They yield no xanthine substances (nuclein bases) as cleavage products. They are not nucleoproteids and therefore they must not be considered together with the gluconucleoproteids (nucleoglucoproteids, or mistaken for them. On pepsin digestion they may, like certain nucleoalbumins, yield pseudonuclein, but they differ from the nucleoalbumins in that they yield a reducing substance on boiling with dilute acid. They differ from the gluconucleoproteids in that they do not, as above mentioned, yield any xanthine bodies.

Only two phosphorized glucoproteids are known at the present time, namely, *ichthulin*, occurring in carp eggs and studied by WALTER [1] and which was considered as a vitellin for a time. Ichthulin has the following composition: C 53.52; H 7.71; N 15.64; S 0.41; P 0.43; Fe 0.10 per cent. In regard to solubilities it is similar to a globulin. WALTER has prepared a reducing substance from the paranuclein of ichthulin which gave a highly crystalline compound with phenylhydrazine.

Another phosphoglucoproteid is *helicoproteid*, obtained by HAMMARSTEN [2] from the glands of the snail Helix pomatia. It has the following composition: C 46.99; H 6.78; N 6.08; S 0.62; P 0.47 per cent. It is converted into a gummy, lævorotatory carbohydrate, called *animal sinistrin*, by the action of alkalies. On boiling with an acid it yields a dextrorotatory reducing substance.

The compound proteid found by SCHULZ and DITTHORN [3] in the spawn of the frog probably belongs to this group, but instead of glucosamine it gives galactosamine on cleavage.

Nucleoproteids. With this name we designate those compound proteids which yield true nucleins (see Chapter V) on pepsin digestion, and on cleavage with dilute caustic alkali yield proteid and nucleic acid.

The nucleoproteids seem to be widely diffused in the animal body. They occur chiefly in the cell-nuclei, but they also often occur in the protoplasm. They may pass into the animal fluids on the destruction of the cells, hence nucleoproteids have also been found in blood-serum and other fluids.

They may be considered as combinations of a proteid nucleus with a side chain, which KOSSEL calls the PROSTHETIC GROUP. This side chain, which contains the phosphorus, may be split off as nucleic acid (see Chapter V) on treatment with alkali. As we have several nucleic acids, it follows that we must have different nucleoproteids, depending upon the nucleic acid united with the proteid. Certain nucleic acids contain a readily split-off sugar (pentose or hexose); others, on the contrary, do not. In the first case we obtain from the corresponding nucleoproteid a reducing sugar on boiling with dilute mineral acid, while in the other case this is not possible. Corresponding to this different behavior we may divide off a special group of nucleoproteids, the gluconucleoproteids or nucleoglucoproteids. Such gluconucleoproteids, yielding pentoses, occur in yeast-cells, and, as it appears, are widely distributed in the animal organism (BLUMENTHAL, GRUND [1]).

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[1] Zeitschr. f. physiol. Chem., 15.

[2] Hammarsten, Pflüger's Arch., 36.

[3] Zeitschr. f. physiol. Chem., 29.

The native nucleoproteids contain a variable but not a high percentage of phosphorus, which HALLIBURTON [2] found to vary between 0.5 per cent and 1.6 per cent. On heating their solutions, as well as by the action of dilute acids, a modification of the compound proteid takes place, and nucleoproteids of strong acid character, poorer in proteid but richer in phosphorus, are formed. The native nucleoproteids have faint acid properties and are insoluble in water,. but their alkali compounds, which are soluble in water, split on heating their solutions into coagulated proteid and a nucleoproteid rich in phosphorus, which remains in solution. In peptic digestion they yield so-called true nuclein, which is also a nucleoproteid poor in proteid. The proteid can be precipitated by acetic acid from its alkali compound, and the precipitate dissolves with more or less readiness in an excess of the acid. A confusion may occur here with nucleoalbumins and also with mucin substances. This confusion may be avoided by warming the body for some time on the water-bath with dilute sulphuric acid, nearly neutralizing the boiling-hot fluid with barium hydrate, filtering as quickly as possible while boiling hot, and testing the filtrate for purine bodies with copper sulphate and bisulphite according to the method given on page 163. Any precipitate formed is examined more closely by the method there given. The nucleoproteids give the color reactions of the proteins, but those which have been investigated are dextrorotatory and not levorotatory (GAMGEE and JONES [3]).

The properties of the various nucleoproteids are given in detail in the various chapters which follow.

III. Albuminoids or Proteinoids.

Under this name we collect into a special group all those protein bodies which cannot be placed in either of the other two groups, although they differ essentially among themselves, and from a chemical standpoint do not show any radical difference from the true protein bodies. The most important and abundant of the bodies belonging to this group are important constituents of the animal skeleton or the cutaneous structure. They occur as a rule in an insoluble state in the organism, and they are distinguished in most cases by a pronounced resistance to reagents which dissolve proteins or to chemical reagents in general.

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[1] Blumenthal, Berlin. klin. Wochenschr., 1897, and Zeitschr. f. klin. Med., **34**; Grund, Zeitschr. f. physiol. Chem., **35**. See also Bendix and Ebstein, Zeitschr. f. allgem. Phys., **2**; Levene and Mandel, Zeitschr. f. physiol. Chem., **47**.

[2] Journ. of Physiol., **18**.

[3] Hofmeister's Beiträge, **4**.

The Keratin Group. Keratin is the chief constituent of the horny structure, of the epidermis, of hair, wool, of the nails, hoofs, horns, feathers, of tortoise-shell, etc., etc. Keratin is also found as neurokeratin (KÜHNE) in the brain and nerves. The shell-membrane of the hen's egg seems also to consist of keratin, and according to NEUMEISTER[1] the organic matrix of the egg-shells of various vertebrate animals belongs in most cases to the keratin group.

It seems that there exist a number of keratins, and these form a special group of bodies. This fact, together with the difficulty in isolating the keratin from the tissues in a pure condition without a partial decomposition, is sufficient explanation for the variation in the elementary composition given below. As examples the analyses of a few tissues rich in keratin and of keratins are given.[2]

	C	H	N	S	O	
Human hair....	50.65	6.36	17.14	5.00	20.85	(v. LAAR)
Nail..........	51.00	6.94	17.51	2.80	21.75	(MULDER)
Neurokeratin...	56.11–58.45	7.26–8.02	11.46–14.32	1.63–2.24	.....	(KÜHNE)
Horn (average)..	50.86	6.94	.....	3.20	.....	(HORBACZEWSKI)
Tortoise-shell...	54.89	6.56	16.77	2.22	19.56	(MULDER)
Shell-membrane.	49.78	6.94	16.43	4.25	22.90	(LINDVALL)

MOHR[3] has determined the quantity of sulphur in various keratin substances. Sulphur is in great part in loose combination, and it is chiefly removed by the action of alkalies (as sulphides), or indeed in part by boiling with water. Combs of lead after long usage become black, and this is due to the action of the sulphur of the hair. On heating keratin with water in sealed tubes to a temperature of 150° C. or higher, it dissolves, with the elimination of sulphuretted hydrogen or mercaptan (BAUER), and the solution contains proteose-like substances (KRUKENBERG) called *atmidkeratin* and *atmidkeratose* by BAUER.[4] Keratin is dissolved by alkalies, especially on warming, producing besides alkali sulphides also proteose substances.

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[1] Kühne and Ewald, Verh. d. naturhistor.-med. Vereins zu Heidelberg (N. F.), **1**; also Kühne and Chittenden, Zeitschr. f. Biologie, **26**; Neumeister, *ibid.*, **31**.

[2] v. Laar, Annal. d. Chem. u. Pharm., **45**; Mulder, Versuch einer allgem. physiol. Chem., Braunschweig, 1844–51; Kühne, Zeitschr. f. Biologie, **26**; Horbaczewski, see Drechsel in Ladenburg's Handwörterbuch d. Chem., **3**; Lindvall, Maly's Jahresbericht, 1881.

[3] Zeitschr. f. physiol. Chem., **20**.

[4] Krukenberg, Untersuch. über d. chem. Bau d. Eiweisskörper, Sitzungsber. d.

Besides the well-known cleavage products such as leucine, tyrosine, aspartic acid, glutamic acid, arginine, and lysine, **Fischer** and **Dörpinghaus**[1] have recently found glycocoll, alanine, α-aminovalerianic acid, α-proline, serine, phenylalanine, and pyrrolidone-carboxylic acid (secondary from glutamic acid) among the cleavage products of horn substances. **Emmerling** claims to have found cystine as a sulphurized cleavage product, but K. **Mörner**[2] was the first to positively prove the abundant occurrence of cystine in the cleavage products. **Mörner** obtained from ox-horn, human hair, and the shell-membrane of the hen's egg 6.8, 13.92, and 7.62 per cent cystine calculated on the basis of the dry substance. From the amount of sulphur split off by alkali, he concludes that, at least in ox-horn and human hair, all the sulphur exists as cystine. **Galimard**[3] was able to get only a qualitative test for cystine in the keratin of the adder eggs. **Suter**, **Mörner**, and **Friedmann**[4] have obtained α-thiolactic acid as a hydrolytic cleavage product of the keratin substances. The last-mentioned investigator was also able to detect thioglycolic acid in the cleavage products of wool.

Bodies occur in the animal kingdom which form to a certain extent intermediate substances between coagulated protein and keratin. C. **Th. Mörner**[5] has detected such a body (*albumoid*) in the tracheal cartilage, which forms a net-like trabecular tissue. This substance appears to be related to the keratins on account of its solubilities and the quantity of the sulphur (lead-blackening) it contains, while according to its solubility in gastric juice it must stand close to the proteins. Another substance, more similar to keratin, is the horny layer in the gizzard of birds. According to J. **Hedenius**[6] this substance is insoluble in gastric or pancreatic juice and acts quite like keratin. It contains only 1 per cent sulphur and yields on decomposition only a very little tyrosine but considerable leucine.

Keratin is amorphous or takes the form of the tissues from which it was prepared. On heating it decomposes and generates an odor of burnt horn. It is insoluble in water, alcohol, or ether. On heating with water to 150–200° C. it dissolves. It also dissolves gradually in caustic alkalies, especially on heating. It is not dissolved by artificial gastric juice or by trypsin solutions. Keratin gives the xanthoproteic reaction, as well as the reaction with **Millon**'s reagent, although the latter is not always typical.

Jenaischen Gesellsch. f. Med. u. Naturwissensch., 1886; Bauer, Zeitschr. f. physiol. Chem., 35.

[1] Zeitschr. f. physiol. Chem., 36, which contains also the older literature.

[2] Mörner, *ibid.*, 34 and 42; Emmerling, Ref. in Chemiker Zeitung, 1894.

[3] Chem. Centralbl. II., 1905.

[4] Suter, Zeitschr. f. physiol. Chem., 20; Mörner, *ibid.*, 42; Friedmann, Hofmeister's Beiträge, 2.

[5] See Maly's Jahresber., 18.

[6] Skand. Arch. f. Physiol., 3.

In the preparation of keratin a finely divided horny structure is treated first with boiling water, then consecutively with diluted acid, pepsin-hydrochloric acid, and alkaline trypsin solution, and, lastly, with water, alcohol, and ether.

Elastin occurs in the connective tissue of higher animals, sometimes in such large quantities that it forms a special tissue. It occurs most abundantly in the cervical ligament (ligamentum nuchæ).

Elastin used to be generally considered as a sulphur-free substance. According to the investigations of CHITTENDEN and HART, it is a question whether or not elastin does not contain sulphur, which is removed by the action of the alkali in its preparation. H. SCHWARZ has been able to prepare an elastin containing sulphur from the aorta by another method, and this sulphur can be removed by the action of alkalies, without changing the properties of the elastin; and recently ZOJA, HEDIN, BERGH, and RICHARDS and GIES[1] have found that elastin contains sulphur. The most trustworthy analyses of elastin from the cervical ligament (Nos. 1 and 2) and from the aorta (No. 3) have given the following results, which compare well with each other:

	C	H	N	S	O	
1.	54.32	6.99	16.75	....	21.94	(HORBACZEWSKI[2])
2.	54.24	7.27	16.70	....	21.79	(CHITTENDEN and HART)
3.	53.96	7.03	16.67	0.38	.....	(H. SCHWARZ)

ZOJA found 0.276 per cent sulphur and 16.96 per cent nitrogen in elastin. HEDIN and BERGH found different quantities of nitrogen in aorta-elastin, depending upon whether HORBACZEWSKI'S or SCHWARZ'S method was used in its preparation. In the first case they found 15.44 per cent nitrogen and 0.55 per cent sulphur, and in the other 14.67 per cent nitrogen and 0.66 per cent sulphur. RICHARDS and GIES found 0.14 per cent sulphur and 16.87 per cent nitrogen in elastin. Abundant leucine, but very little tyrosine, some glycocoll, and perhaps aminovalerianic acid, but no aspartic acid or glutamic acid, used to be considered amongst the hydrolytic cleavage products of elastin. ABDERHALDEN and SCHITTENHELM[3] have obtained glycocoll 25.75; leucine 21.38; alanine 6.58; phenylalanine 3.89; α-proline 1.74; glutamic acid 0.76, and aminovalerianic acid 1.0 per cent. The three hexone bases have been obtained, but only in very small amounts, so that the basic nitrogen represents only 3.34 per cent of the total nitrogen (RICHARDS and GIES). This fact and the very low sulphur content make it questionable whether the elastin is a unit body.

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[1] Chittenden and Hart, Zeitschr. f. Biologie, 25; Schwarz, Zeitschr. f. physiol. Chem., 18; Zoja, *ibid.*, 23; Bergh, *ibid.*, 25; Hedin, *ibid.*; Richards and Gies, Amer. Journ. of Physiol., 7.

[2] Zeitschr. f. physiol. Chem., 6.

[3] *Ibid.*, 41.

On putrefaction by anaerobic micro-organisms, ZOJA found carbon dioxide, hydrogen, methane, mercaptan, butyric acid, valerianic acid, ammonia, and possibly also phenylpropionic acid and aromatic oxyacids. Indol and skatol have not been found in putrefaction,[1] but SCHWARZ, on the contrary, obtained indol, skatol, benzene, and phenols on fusing aorta-elastin with caustic potash. On heating with water in closed vessels, on boiling with dilute acids, or by the action of proteolytic enzymes, the elastin dissolves and splits into two chief products, called by HORBACZEWSKI *hemielastin* and *elastinpeptone*. According to CHITTENDEN and HART, these products correspond to two proteoses designated by them *protoelastose* and *deuteroelastose*. The first is soluble in cold water and separates out on heating, and its solution is precipitated by mineral acids as well as by acetic acid and potassium ferrocyanide. The aqueous solution of the other does not become cloudy on heating, and is not precipitated by the above-mentioned reagents. According to RICHARDS and GIES, elastoses, especially protoelastoses, and true peptone are formed, the latter only to a slight extent.

Pure elastin when dry is a yellowish-white powder; in the moist state it appears like yellowish-white threads or membranes. It is insoluble in water, alcohol, or ether, and shows a resistance toward the action of chemical reagents. It is not dissolved by strong caustic alkalies at the ordinary temperature and only slowly at the boiling temperature. It is very slowly attacked by cold concentrated sulphuric acid, but it is relatively easily dissolved on warming with strong nitric acid. Elastins of different origins act differently with cold concentrated hydrochloric acid; for instance, elastin from the aorta dissolves readily therein, while elastin from the ligamentum nuchæ, at least from old animals, dissolves with difficulty. Elastin is more readily dissolved by warm concentrated hydrochloric acid. It responds to the xanthoproteic reaction and to that with MILLON'S reagent.

On account of its great resistance to chemical reagents, elastin may be prepared (best from the ligamentum nuchæ) in the following way: First boil with water, then with 1 per cent caustic potash, then again with water, and lastly with acetic acid. The residue is treated with cold 5 per cent hydrochloric acid for twenty-four hours, carefully washed with water, boiled again with water, and then treated with alcohol and ether.

In regard to the methods used by SCHWARZ and by RICHARDS and GIES, which are somewhat different, we refer to the original publications.

Collagen, or gelatine-forming substance, occurs very extensively in vertebrates. The flesh of cephalopods is also said to contain collagen.[2] Collagen is the chief constituent of the fibrils of the connective tissue and (as ossein) of the organic substances of the bony structure. It also occurs in the cartilaginous tissues as chief constituent; but it is here mixed with other substances, producing what was formerly called chondrigen. Collagen from different tissues has not quite the same composition, and probably there are several varieties of collagen.

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[1] See Wälchli, Journ. f. prakt. Chem. (N. F.), **17.**

[2] Hoppe-Seyler, Physiol. Chem., p. 97.

By continued boiling with water (more easily in the presence of a little acid) collagen is converted into gelatine. HOFMEISTER[1] found that gelatine on being heated to 130° C. is again transformed into collagen; and this last may be considered as the anhydride of gelatine. Collagen and gelatine have about the same composition.[2]

	C	H	N	S	O	
Collagen	50.75	6.47	17.86	24.92		(HOFMEISTER)
Gelatine (commercial)	49.38	6.80	17.97	0.7	25.13	(CHITTENDEN)
Gelatine from tendons	50.11	6.56	17.81	0.26	25.26	(VAN NAME)
Gelatine from ligaments	50.49	6.71	17.90	0.57	24.33	(RICHARDS and GIES)
Fish glue	48.69	6.76	17.68	....	.....	(FAUST)

Gelatines of different origin show a somewhat variable composition, which seems to indicate the occurrence of different collagens. It is difficult to say whether the variable content of sulphur is due to a contamination with a substance rich in sulphur or to a splitting off of loosely combined sulphur during the purification. C. MÖRNER[3] has prepared a typical gelatine containing only 0.2 per cent of sulphur by a method which eliminated any possible changes due to reagents.

SADIKOFF[4] has prepared gelatines by various methods from tendons and from cartilage. Those from tendons, some of which were prepared after previous tryptic digestion, some after treatment with 0.25 per cent caustic potash, and some after treatment with sodium hydroxide and then carbonate, showed somewhat different physical properties among each other, but had about the same elementary composition, with 0.34–0.526 per cent sulphur. SADIKOFF seems to think that the gelatines prepared up to this time were perhaps not unit bodies but were possibly mixtures. The bodies prepared by SADIKOFF from cartilage he calls *gluteins*, because they were essentially different from the other gelatines or glutins. They were poorer in carbon and nitrogen, 17.7 to 17.87 per cent, but somewhat richer in sulphur, 0.53–0.712 per cent, than the tendon glutin. The gluteins differ also from the glutins in that on boiling with a mineral acid they have a faint reducing action, and also in that they give a color reaction with phloroglucin-hydrochloric acid. The glutins differ from the gluteins by a different behavior with certain salts.

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[1] Zeitschr. f. physiol. Chem., 2.

[2] Hofmeister, l. c.; Chittenden and Solley, Journ. of Physiol., 12; van Name, Journ. of Exper. Med., 2; Richards and Gies, Amer. Journ. of Physiol., 8; Faust, Arch. f. exp. Path. u. Pharm., 41.

[3] Zeitschr. f. physiol. Chem., 28.

[4] *Ibid.*, 39 and 41.

The decomposition products of the collagens are the same as those of the gelatines. Besides the leucine, glycocoll, aspartic acid, and glutamic acid found by the earlier investigators as hydrolytic cleavage products, E. FISCHER and collaborators[1] have obtained alanine, phenylalanine, and α-proline. Gelatine does not give any tyrosine, but does yield considerable glycocoll (16.5 per cent according to E. FISCHER), which because of its sweetish taste has received the name gelatine-sugar. SKRAUP[2] has obtained on the hydrolytic cleavage of gelatine a crystalline acid having the formula $C_{12}H_{25}N_5O_{10}$, which he calls *glutinic acid*. Gelatine yields considerable basic nitrogen, according to HAUSMANN[3] 35.83 per cent of the total nitrogen. DRECHSEL and FISCHER found lysine; HEDIN, KOSSEL and KUTSCHER[4] found also arginine, which amounted to 9.3 per cent (KOSSEL and KUTSCHER). On putrefaction gelatine gives neither tyrosine, indol, nor skatol. According to SELTRENNY[5] it yields phenylpropionic acid and phenylacetic acid. The aromatic group in gelatine is therefore, as directly shown by FISCHER (see above) and also by SPIRO,[6] represented by phenylalanine.

On the oxidation of gelatine with potassium permanganate, SEEMANN obtained, besides volatile fatty acids (formic, acetic, butyric acids), benzoic acid, oxalic acid, succinic acid, oxaluramide and probably also oxaluric acid. ZICKGRAF[7] produced guanidine from the arginine.

Collagen is insoluble in water, salt solutions, and dilute acids and alkalies, but it swells up in dilute acids. By continued boiling with water it is converted into gelatine. It is dissolved by the gastric juice and also by the pancreatic juice (trypsin solution) when it has previously been treated with acid or heated with water above 70° C.[8] By the action of ferrous sulphate, corrosive sublimate, or tannic acid, collagen shrinks greatly. Collagen treated by these bodies does not putrefy, and tannic acid is therefore of great importance in the preparation of leather.

Gelatine or glutin is colorless, amorphous, and transparent in thin layers. It swells in cold water without dissolving. It dissolves in warm water, forming a sticky liquid, which solidifies on cooling when sufficiently concentrated. As PAULI and RONA[9] have shown, various bodies may have a different influence upon the gelatinization-point of a gelatine solution;

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[1] Fischer, Levene and Aders, Zeitschr. f. physiol. Chem., **35**. In regard to the older researches, see O. Cohnheim, Chemie der Eiweisskörper, 2. Aufl., 1904.

[2] Monatshefte f. Chem., **26**.

[3] Zeitschr. f. physiol. Chem., **27**.

[4] Drechsel, Arch. f. Anat. u. Physiol., 1891; Hedin, Zeitschr. f. physiol. Chem., **21**; Kossel and Kutscher, *ibid.*, **31**.

[5] Monatshefte f. Chem., **10**.

[6] Hofmeister's Beiträge, **1**.

[7] Seemann, Zeitschr. f. physiol. Chem., **44**; Zickgraf, *ibid.*, **41**.

[8] Kühne and Ewald, Verh. d. Naturhist. Med. Vereins in Heidelberg, 1877, **1**.

[9] Hofmeister's Beiträge, **2**.

thus certain substances such as sulphates, citrates, acetates, and glycerine may accelerate, while the chlorides, chlorates, bromides, alcohol, and urea retard this power.

Gelatine solutions are not precipitated on boiling, nor by mineral acids, acetic acid, alum, basic lead acetate, nor metallic salts in general. A gelatine solution acidified with acetic acid may be precipitated by potassium ferrocyanide on carefully adding the reagent. Gelatine solutions are precipitated by tannic acid in the presence of salt; by acetic acid and common salt in substance; mercuric chloride in the presence of HCl and NaCl; metaphosphoric acid and phosphomolybdic acid in the presence of acid; and lastly also by alcohol, especially when neutral salts are present. Gelatine solutions do not diffuse. Gelatine gives the biuret reaction, but not ADAMKIEWICZ'S. It gives MILLON'S reaction and the xanthoproteic reaction so faintly that they probably occur from impurities consisting of proteids. According to C. MÖRNER, pure gelatine gives a beautiful MILLON'S reaction, if not too much reagent is added. In the other case no reaction or only a faint one is obtained.

By continued boiling with water gelatine is converted into a non-gelatinizing modification called β-glutin by NASSE. According to NASSE and KRÜGER the specific rotatory power is hereby reduced from −167.5° to about −136°.[1] On prolonged boiling with water, especially in the presence of dilute acids, also in the gastric or tryptic digestion, the gelatine is transformed into gelatine proteoses, so-called *gelatoses* and *gelatine peptones*, which diffuse more or less readily.

According to HOFMEISTER two new substances, *semiglutin* and *hemicollin*, are formed. The former is insoluble in alcohol of 70–80 per cent and is precipitated by platinum chloride. The latter, which is not precipitated by platinum chloride, is soluble in alcohol. CHITTENDEN and SOLLEY[2] have obtained in the peptic and tryptic digestion a *proto-* and a *deuterogelatose*, besides a true peptone. The elementary composition of these gelatoses does not essentially differ from that of the gelatine.

According to LEVENE the proto- as well as the deuterogelatoses yield a larger amount of glycocoll, as much as 20.3 per cent, than the gelatine itself. On prolonged tryptic digestion a further demolition takes place, so that the peptone yields only about the same amount of glycocoll as the gelatine. Some leucine and, as it appears, also some glutamic acid and phenylalanine are split off. Quite a considerable splitting off of NH_3 also takes place (LEVENE and STOOKEY).[3] PAAL[4] has prepared gelatine-peptone hydrochlorides from gelatine by the action of dilute hydrochloric acid. These salts are partly soluble in ethyl and methyl alcohol, and partly insoluble therein. The peptones obtained from these salts contain less carbon and more hydrogen than the gelatine from which they originated, showing that hydration has taken place. The molecular weight of the gelatine peptone as determined by PAAL, by RAOULT's cryoscopic method, was 200 to 352, while that for gelatine was 878 to 960. The gelatine peptones isolated by SIEGFRIED and his pupils SCHEERMESSER[1] and KRÜGER, and which have already been mentioned, are of the greatest interest.

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[1] Nasse and Krüger, Maly's Jahresber., **19**, p. 29. In regard to the rotation of β-glutin, see Framm, Pflüger's Arch., **68**.

[2] Hofmeister, l. c.; Chittenden and Solley, l. c.

[3] Levene, Zeitschr. f. physiol. Chem., **37**; Levene and Stookey, *ibid.*, **41**.

[4] Ber. d. deutsch. chem. Gesellsch., **25**.

Collagen (contaminated with mucoid) may be obtained from bones by extracting them with hydrochloric acid (which dissolves the earthy phosphates) and then carefully washing the acid out with water. It may be obtained from tendons by extracting with lime-water or dilute alkali (which dissolve the proteids and mucin) and then thoroughly washing with water. Gelatine is obtained by boiling collagen with water. The finest commercial gelatine always contains a little proteid, which may be removed by allowing the finely divided gelatine to swell up in water and thoroughly extracting with large quantities of fresh water. Then dissolve in warm water and precipitate with alcohol.

Collagen may also be purified from proteids, as suggested by VAN NAME, by digesting with an alkaline trypsin solution or by extracting the gelatine for many days with 1–5 p. m. caustic potash, as suggested by C. MÖRNER. The typical properties of gelatine are not changed by this.

Chondrin or cartilage gelatine is only a mixture of gelatine with the specific constituents of the cartilage and their transformation products.

Reticulin. The reticular tissues of the lymphatic glands contain a variety of fibres which have also been found by MALL in the spleen, intestinal mucosa, liver, kidneys, and lungs. These fibres consist of a special substance, reticulin, investigated by SIEGFRIED.[2]

Reticulin has the following composition: C 52.88; H 6.97; N 15.63; S 1.88; P 0.34; ash 2.27 per cent. The phosphorus occurs in organic combination. It yields no tyrosine on cleavage with hydrochloric acid. It yields, on the contrary, sulphuretted hydrogen, ammonia, lysine, arginine, and aminovalerianic acid. On continued boiling with water, or more readily with dilute alkalies, reticulin is converted into a body which is precipitated by acetic acid, and at the same time phosphorus is split off.

Reticulin is insoluble in water, alcohol, ether, lime-water, sodium carbonate, and dilute mineral acids. It is dissolved, after several weeks, on standing with caustic soda at the ordinary temperature. Pepsin-hydrochloric acid or trypsin does not dissolve it. Reticulin responds to the biuret, xanthoproteic, and ADAMKIEWICZ's reactions, but not to MILLON's reagent.

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[1] Zeitschr. f. physiol. Chem., **37** and **41**; Krüger, l. c. See foot-note 3, p. 57.

[2] Mall, Abhandl. d. math.-phys. Klasse d. Kgl. sächs. Gesellsch. d. Wiss., 1891; Siegfried, Ueber die chem. Eigensch. der retikulirten Gewebe, Habil.-Schrift, Leipzig, 1892.

According to Tebb reticulin is only a somewhat changed, impure collagen, but this is disputed by Siegfried.[1]

It may be prepared as follows, according to Siegfried: Digest intestinal mucosa with trypsin and alkali. Wash the residue, extract with ether, and digest again with trypsin and then treat with alcohol and ether. On careful boiling with water the collagen present either as contamination or as a combination with reticulin is removed. The thoroughly boiled residue consists of reticulin.

Ichthylepidin is an organic compound, so called by C. Mörner,[2] which occurs with collagen in fish-scales and forms about ⅕ of their organic substance. This compound, with 15.9 per cent nitrogen and 1.1 per cent sulphur, stands on account of its properties rather close to elastin. It is insoluble in cold and hot water, as well as in dilute acids and alkalies at the ordinary temperature. On boiling with these it dissolves. Pepsin-hydrochloric acid, as well as an alkaline trypsin solution, also dissolves it. It responds beautifully with Millon's reagent, the xanthoproteic reaction, and the biuret test. At least a part of the sulphur is split off by the action of alkali.

As **skeletins**, Krukenberg[3] has designated a number of nitrogenized substances which form the skeletal tissue of various classes of invertebrates. These substances are *chitin*, *spongin*, *conchiolin*, *cornein*, and *fibroin* (silk). Of these chitin does not belong to the protein substances, and fibroin (silk) is hardly to be classed as a skeletin. Only those so-called skeletins will be discussed that actually belong to the protein group.

Spongin forms the chief mass of the ordinary sponge. It gives no gelatine. On boiling with acids, according to the older statements, it yields leucine and glycocoll, but not tyrosine. Zalocostas claims to have found tyrosine and also amino-isovalerianic acid and glucalanine ($C_6H_{12}N_2O_4$). After Hundeshagen had shown the occurrence of iodine and bromine in organic combination in different sponges and designated the albumoid containing iodine, *iodospongin*, Harnack[4] later isolated from the ordinary sponge, by cleavage with mineral acids, an iodospongin which contained about 9 per cent iodine and 4.5 per cent sulphur. On the hydrolysis of spongin Abderhalden and Strauss[5] obtained abundance of glutamic acid, 18.1, and glycocoll, 13.9 per cent, also leucine, 7.5, α-proline, 6.3, and aspartic acid, 4.1 per cent. Very remarkable was the fact that neither tyrosine nor phenylalanine could be detected. Strauss[6] has obtained *sponginoses* of various kinds from spongin by dilute acids. The heterosponginose contained the greater part of the iodine and sulphur, while the deuterosponginose contained the carbohydrate groups. Iodospongin is considered as a derivative of the heterosponginose.

Conchiolin is found in the shells of mussels and snails and also in the egg-shells of these animals. It yields, according to Wetzel,[7] glycocoll, leucine, and abundance of tyrosine. The quantity of diamino-nitrogen amounts to 8.7 per cent and the amide nitrogen 3.47 per cent (from the shell of pinna). The **Byssus** contains a substance, closely related to conchiolin, which is soluble with difficulty. **Cornein** forms the axial system of the Antipathes and Gorgonia. It gives leucine and a crystallizable substance, *cornicrystalline*. According to DRECHSEL the axial system of the Gorgonia cavolini contains nearly 8 per cent of the dry substance as iodine. The iodine occurs in organic combination with an iodized albumoid, *gorgonin*, which is a cornein. DRECHSEL obtained leucine, tyrosine, lysine, ammonia, and an iodized amino-acid, *iodogorgonic acid*, as cleavage products of gorgonin. According to WHEELER and JAMIESON[1] iodogorgonic acid is diiodotyrosine, probably 3,5-diiodotyrosine, $C_6H_2(CH_2CH(NH_2)COOH)(OH)I_2$, and was prepared by them by the action of iodine upon tyrosine and alkali. HENZE[2] could obtain this acid only in very small quantities, and by acid cleavage of gorgonin he obtained the three hexone bases, abundance of tyrosine, and very little leucine. On cleavage with barium hydrate he obtained only lysine besides tyrosine and glycocoll in larger amounts.

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[1] Tebb, Journ. of Physiol., 27; Siegfried, *ibid.*, 28.

[2] Zeitschr. f. physiol. Chem., 24 and 37. See also Green and Tower, *ibid.*, 35.

[3] Grundzüge einer vergl. Physiol. d. thier. Gerüstsubst., Heidelberg, 1885.

[4] Zalocostas, Compt. rend., 107; Hundeshagen, Maly's Jahresber., 25; Harnack, Zeitschr. f. physiol. Chem., 24.

[5] Zeitschr. f. physiol. Chem., 48.

[6] Biochem. Centralbl., 3.

[7] Zeitschr. f. physiol. Chem., 29, and Centralbl. f. Physiol., 13, 113.

Fibroin and **sericin** are the two chief constituents of raw silk. By the action of boiling water the sericin (silk gelatine) dissolves and can be obtained by a method suggested by BONDI,[3] while the more difficultly soluble fibroin remains undissolved in the shape of the original fibre. The sericin, whose sufficiently concentrated hot solution gelatinizes on cooling, is precipitated by mineral acids, several metallic salts, and by acetic acid and potassium ferrocyanide. As cleavage products E. FISCHER and SKITA obtained alanine, serine, very little glycocoll, tyrosine, arginine, and probably also lysine. Leucine had been found previously. From fibroin they obtained, besides the previously known cleavage products, glycocoll, tyrosine, and alanine (WEYL[4]), also leucine, phenylalanine, serine, α-proline (FISCHER), and a small amount of arginine. The chief products were glycocoll, 36 per cent, alanine, 21 per cent, and tyrosine, 10 per cent. The composition of the above-mentioned albuminoids is as follows:[5]

	C	H	N	S	
Conchiolin (from the shells of pinna)...	52.70	6.54	16.60	0.85	(WETZEL)
“ (from snail eggs).........	50.92	6.88	17.86	0.31	(KRUKENBERG)
Spongin..........................	46.50	6.30	16.20	0.50	(CROOCKEWITT)
“	48.75	6.35	16.40	....	(POSSELT)
Cornein..........................	48.96	5.90	16.81	....	(KRUKENBERG)
Fibroin..........................	48.23	6.27	18.31	....	(CRAMER)
“	48.30	6.50	19.20	....	(VIGNON)
Sericin..........................	44.32	6.18	18.30	....	(CRAMER)
“	44.50	6.32	17.14	....	(BONDI)

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[1] Amer. Chem. Journ., **33**.

[2] Drechsel, Zeitschr. f. Biologie, **33**; Henze, Zeitschr. f. physiol. Chem., **38**.

[3] Zeitschr. f. physiol. Chem., **34**.

[4] Fischer and Skita, *ibid.*, **33**; Fischer, *ibid.*, **39**; Weyl, Ber. d. d. chem. Gesellsch., **21**.

[5] Krukenberg, Ber. d. d. chem. Gesellsch., **17** and **18**, and Zeitschr. f. Biologie, **22**; Croockewitt, Annal. d. Chem. u. Pharm., **48**; Posselt, *ibid.*, **45**; Cramer, Journ. f. prakt. Chem., **96**; Vignon, Compt. rend., **115**; Wetzel, l. c., and Bondi, l. c.

Appendix to Chapter II.

HYDROLYTIC CLEAVAGE PRODUCTS OF THE PROTEIN SUBSTANCES.

1. Monamino-acids.

Glycocoll (aminoacetic acid), $C_2H_5NO_2=\frac{CH_2(NH_2)}{COOH}$, also called glycine or gelatine sugar, is found in the muscles of the invertebrates, but has chief interest as a hydrolytic decomposition product of protein bodies, especially gelatine, fibroin, and spongin, as well as of hippuric acid and glycocholic acid. It is also formed in the decomposition of uric acid, xanthine, guanine, and adenine.

Glycocoll has been most abundantly obtained thus far from the protein substances fibroin[1] (36 per cent), elastin[2] (25.75 per cent), gelatine and gelatoses[3] (16.5 and 20.3 per cent respectively).

Glycocoll forms colorless, often large, hard rhombic crystals or four-sided prisms. The crystals have a sweet taste and dissolve readily in cold water (4.3 parts). It is insoluble in alcohol and ether and dissolves with difficulty in warm alcohol. Glycocoll combines with acids and alkalies. With the latter compounds we must mention those with copper and silver. Glycocoll dissolves cupric hydrate in alkaline liquids but does not reduce at boiling heat. A boiling-hot solution of glycocoll dissolves freshly precipitated cupric hydrate, forming a blue solution, which, in proper concentration, deposits blue needles of copper glycocoll on cooling. The compound with hydrochloric acid is readily soluble in water but less soluble in alcohol.

SÖRENSEN[4] finds that phosphotungstic acid does not precipitate glycocoll from dilute solutions but only from concentrated ones. By the action of gaseous HCl upon glycocoll in absolute alcohol, beautiful crystals are obtained of the hydrochloride of glycocoll ethyl ester, which melts at 144° C. and from which the glycocoll ethyl ester can be obtained by the method suggested by E. FISCHER[5] for the separation of glycocoll from the other amino-acids. On shaking with benzoyl chloride and caustic soda, hippuric acid is formed, and this is also made use of in different ways in detecting and isolating glycocoll (CH. FISCHER, GONNERMANN, SPIRO[6]). The melting-point of glycocoll-β-naphthalenesulphonate is 156° (corr. 159°), of glycocoll 4-nitrotoluene-2-sulphonate 177.5° (corr. 178°), of the phenylisocyanate compound 195°, and of the α-naphthylisocyanate compound 190.5–191.5°.

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[1] Fischer and Skita, Zeitschr. f. physiol. Chem., 33.

[2] Abderhalden and Schittenhelm, *ibid.*, 41.

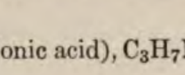
[3] Fischer. Levene and Aders, *ibid.*, 35; Levene, *ibid.*, 37 and 41.

[4] Meddelelser, fraa Carlsberg-laboratoriet, 6, 1905.

[5] Ber. d. d. chem. Gesellsch., 34.

[6] Ch. Fischer, Zeitschr. f. physiol. Chem., 19; Spiro, *ibid.*, 28; Gonnermann, Pflüger's Arch., 59.

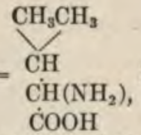
Glycocoll can be best prepared from hippuric acid by boiling it with 4 parts dilute sulphuric acid (1:6) for ten to twelve hours. After cooling the benzoic acid is removed, the filtrate concentrated, the remaining benzoic acid removed by extracting with ether, the sulphuric acid precipitated by $BaCO_3$, and the filtrate evaporated to the point of crystallization. (In regard to its preparation from protein substances see below.)



Alanine (α-aminopropionic acid), $C_3H_7NO_2=\overset{CH_3}{\underset{COOH}{CH(NH_2)}}$, was first obtained by Weyl as a cleavage product of fibroin. This *d*-alanine has been isolated by E. Fischer and his collaborators[1] still more abundantly from fibroin (21 per cent) and also from sericin (5 per cent), horn substance (1.20 per cent), gelatine (0.8 per cent), hæmoglobin (2.87 per cent), and elastin[2] (6.58 per cent).

Alanine has a sweet taste, is readily soluble in water, and dissolves cupric hydrate on boiling, producing copper alanine, which has a deep blue color. The specific rotation of the hydrochloride (9–10 per cent solution) is $(\alpha)_D = +10.3°$. In regard to the synthetical preparation of *i*-alanine, its separation as the benzoyl compound, and the preparation of *i*-alanine ethyl ester we must refer to E. Fischer.[3]

The *d*-alanine-β-naphthalenesulphonate melts at 78–80° (79–81° corr.), the racemic alanine-4-nitrotoluene-2-sulphonate at 96° (uncorr.), the phenylisocyanate compound at 168°, and the α-naphthylisocyanate compound at 198° C.



Aminovalerianic acid, $C_5H_{11}NO_2 = \begin{array}{c}CH_3CH_3\\ \diagdown\diagup\\ CH\\ CH(NH_2),\\ COOH\end{array}$ has been detected several times among the cleavage products of protein substances. Kossel and Dakin[4] obtained 4.3 per cent from salmine. The acid isolated by E. Fischer from horn substance (5.70 per cent) and casein, as well as that obtained by Schulze and Winterstein[5] from lupin sprouts, seems to be dextrorotatory α-aminovalerianic acid. The copper salt of aminovalerianic acid is, according to Schulze and Winterstein,[6] readily soluble in methyl alcohol.

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[1] Weyl, Ber. d. d. chem. Gesellsch., **21**; Fischer and Skita, Zeitschr. f. physiol. Chem., **33**; Fischer and Dörpinghaus, *ibid.*, **36**; Fischer, Levene and Aders, *ibid.*, **35**; Fischer and Abderhalden, *ibid.*, **36**.

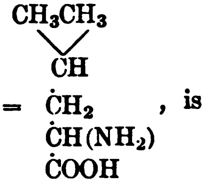
[2] Abderhalden and Schittenhelm, Zeitschr. f. physiol. Chem., **41**.

[3] Ber. d. d. chem. Gesellsch., **32** and **34**.

[4] Zeitschr. f. physiol. Chem., **41**.

[5] Fischer, *ibid.*, **36** and **33**; Schulze and Winterstein, *ibid.*, **35**.

[6] *Ibid.*, **45**.

Leucine (aminocaproic acid, or, more correctly, α-aminoisobutylacetic acid), $C_6H_{13}NO_2$ =  , is produced from protein substances in their hydrolytic cleavage by proteolytic enzymes, by boiling with dilute acids or alkalies or by fusing with alkali hydrates, and by putrefaction. Because of the ease with which leucine (and tyrosine) are formed in the decomposition of protein substances, it is difficult to decide positively whether these bodies when found in the tissues are constituents of the living body or are to be considered only as decomposition products formed after death. Leucine, it seems, has been found as a normal constituent of the pancreas and its secretion, in the spleen, thymus, and lymph glands, in the thyroid gland, in the salivary glands, in the kidneys and in the liver. It also occurs in the wool of sheep, in dirt from the skin (inactive epidermis), and between the toes, and its decomposition products have the disagreeable odor of the perspiration of the feet. It is found pathologically in atheromatous cysts, ichthyosis scales, pus, blood, liver, and urine (in diseases of the liver and in phosphorus poisoning). Leucine occurs often in invertebrates and also in the plant kingdom. On hydrolytic cleavage various protein substances yield different amounts of leucine. ERLENMEYER and SCHÖFFER obtained 36-45 per cent of leucine from the cervical ligament, ABDERHALDEN and SCHITTENHELM 21.38 per cent from elastin, COHN 32 per cent from casein, and NENCKI 1.5-2 per cent from gelatine. E. FISCHER and ABDERHALDEN obtained 20 per cent of leucine from hæmoglobin, FISCHER and DÖRPINGHAUS 18.3 per cent from horn substance, NENCKI 1.5-2 per cent from gelatine, and FISCHER and SKITA 1.5 per cent from fibroin.[1]

Leucine occurs, like other monamino-acids, in the *l*-, *d*-, and *i*-modifications. The leucine obtained by cleavage of protein substances is generally levorotatory in watery solution and dextrorotatory *l*-leucine in acid solution. The leucine prepared synthetically by HÜFNER[2] from isovaleraldehyde, ammonia, and hydrocyanic acid is optically inactive. Inactive leucine may also be prepared, as shown by E. SCHULZE and BOSSHARD,[3] by the cleavage of proteins with baryta at 160-180° C., or by heating ordinary leucine with baryta-water to the same temperature. The levorotatory modification may be formed from the inactive leucine by the action of Penicillum glaucum. On benzoylating *i*-leucine we obtain *i*-benzoylleucine, from whose cinchonine and quinidine salts first *d*- and then *l*-benzoylleucine are prepared, and then by hydrolytic cleavage *d*- and *l*-leucine may be obtained (E. Fischer). On oxidation the leucines yield the corresponding oxyacids (leucinic acids). Leucine is decomposed on heating, evolving carbon dioxide, ammonia, and amylamine. On heating with alkalies, as also in putrefaction, it yields valerianic acid and ammonia.

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[1] Erlenmeyer and Schöffer, cited from Maly, Chem. d. Verdauungssäfte, in Hermann's Handb. d. Physiol., 5, Theil 2, p. 209; Abderhalden and Schittenhelm, Zeitschr. f. physiol. Chem., 41; Cohn, *ibid.*, 22; Nencki, Journ. f. prakt. Chem. (N. F.), 15; Fischer and his collaborators, see p. 84, foot-note 1.

[2] Journ. f. prakt. Chem. (N. F.), 1.

[3] See Zeitschr. f. physiol. Chem., 9 and 10.

Leucine crystallizes when pure in shining, white, very thin plates, usually forming round knobs or balls, either appearing like hyaline, or with alternating light and dark concentric layers which consist of radial groups of crystals. By slow heating, leucine melts and sublimes in white, woolly flakes, which are similar to sublimed zinc oxide. At the same time an odor of amylamine is developed. Quickly heated in a closed capillary tube, it melts with decomposition at 293–295°.

Leucine, as obtained from animal fluids and tissues, is very easily soluble in water and rather easily in alcohol. Pure leucine is soluble with difficulty. Pure *l*- and *d*-leucine dissolve in 40–46 parts water, more readily in hot alcohol, but with difficulty in cold alcohol. The *i*-leucine is much less soluble. According to Habermann and Ehrenfeld[1] 100 parts of boiling glacial acetic acid dissolve 29.23 parts of leucine. The specific rotation of the ordinary leucine, dissolved in hydrochloric acid, is about $(\alpha)_D = +17.5°$.

The solution of leucine in water is not, as a rule, precipitated by metallic salts. The boiling-hot solution may, however, be precipitated by a boiling-hot solution of copper acetate, and this fact is made use of in separating leucine from other substances. If the solution of leucine is boiled with sugar of lead and then ammonia be added to the cooled solution, shining crystalline leaves of leucine-lead oxide separate. Leucine dissolves cupric hydrate, but does not reduce on boiling.

Leucine is readily soluble in alkalies and acids. It gives crystalline compounds with mineral acids. If leucine hydrochloride is boiled with alcohol containing 3–4 per cent HCl, long narrow crystalline prisms of leucine ethyl ester hydrochloride, melting at 134° C., are formed (Röhmann). The same is produced by the action of gaseous HCl upon leucine in alcohol, and the free ethyl ester can be obtained from this by the method suggested by E. Fischer.[2] This ester can be separated from the other amino-acid esters by distillation. The pure leucine can be prepared from the ester by boiling with water for a long time. The picrate of the leucine ester melts at 128° C. The phenylisocyanate compound of *i*-leucine melts at 165° C. and its anhydride at 125° C. The α-naphthylisocyanate compound melts at 163.5° and *l*-leucine β-naphthalenesulphonate melts at 67° (corr. 68°).

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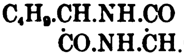
[1] Zeitschr. f. physiol. Chem., **37**.

[2] Röhmann, Ber. d. d. chem. Gesellsch., **30**; E. Fischer, *ibid.*, **34**.

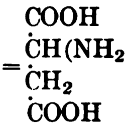
Leucine is recognized by the appearance of balls or knobs under the microscope, by its action when heated (sublimation test), and by its compounds, especially the hydrochloride and picrate of the ethyl ester and the phenylisocyanate compound of the racemic leucine obtained by heating with baryta-water, the α-naphthylisocyanate compound and the leucine β-naphthalenesulphonate. Leucine must first be isolated before it can be detected, and this is best done by preparing the ethyl ester and then distilling it.

Leucinimide, $C_{12}H_{22}N_2O_2 =$  $\begin{array}{l} C_4H_9.CH.NH.CO \\ CO.NH.CH.C_4H_9 \end{array}$, was first obtained by Ritthausen in the hydrolytic cleavage products on boiling proteins with acids, and subsequently by R. Cohn. Salaskin[1] obtained it in the peptic and tryptic digestion of hæmoglobin. As an anhydride of leucine (2.5-diacipiperazine) it is probably formed by a secondary change, from leucine.

It crystallizes in long needles and sublimes readily. The melting-point has not been found constant in the different cases. The leucinimide (3.6-diisobutyl-2.5-diacipiperazine) prepared synthetically by E. Fischer[2] from leucine ethyl ester melted at 271° C.

Isoleucine, an isomer of leucine, has recently been discovered by F. Ehrlich, but its constitution is still unknown. Ehrlich first isolated it from the mother-liquor after removing the sugar from molasses, and found it also on the hydrolysis of several proteins, and considers it as regularly associated with ordinary leucine. Winterstein and Pantanelli obtained it on the hydrolysis of the protein of lupin seeds, and it has also been found by Schulze and Winterstein[3] in sprouts.

Isoleucine is more soluble in water than *l*-leucine (1 : 25.8). It is dextrorotatory in aqueous as well as in acid solutions and in the presence of hydrochloric acid it acts more than twice as strongly as ordinary leucine. In aqueous solution the specific rotation is $(\alpha)_D = +9.74°$, in hydrochloric-acid solution $= +36.8°$. Isoleucine melts at 280°, and the benzoyl compound has a melting-point of 116–117°. Its copper salt is rather soluble in water and, like the copper salt of aminovalerianic acid, is readily soluble in methyl alcohol.

Aspartic Acid (aminosuccinic acid), $C_4H_7NO_4 =$  $\begin{array}{l} COOH \\ CH(NH_2) \\ CH_2 \\ COOH \end{array}$, has been obtained on the cleavage of protein substances by proteolytic enzymes as well as by boiling them with dilute mineral acids. Hlasiwetz and Habermann obtained 23.8 per cent from ovalbumin and 9.3 per cent from casein, although the product was not quite pure. E. Fischer and his co-workers[1] obtained 3.29 per cent aspartic acid from hæmoglobin, 2.50 per cent from horn substance, and 0.56 per cent from gelatine. This acid also occurs in secretions of sea-snails (HENZE[2]) and is very widely diffused in the vegetable kingdom as the amide ASPARAGINE (aminosuccinic-acid amide), which seems to be of the greatest importance in the development and formation of the proteins in the plants.

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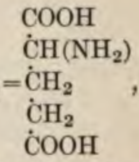
[1] Ritthausen, Die Eiweisskörper der Getreidearten, etc., Bonn, 1872; R. Cohn, Zeitschr. f. physiol. Chem.. 22 and 29; Salaskin, *ibid.*, 32.

[2] Ber d. d. chem. Gesellsch., 34.

[3] Felix Ehrlich. *ibid.*, 37; Winterstein and Pantanelli, Zeitschr. f. physiol. Chem., 45; Schulze and Winterstein *ibid.*. 45

Aspartic acid dissolves in 256 parts water at 10° C. and in 18.6 parts boiling water, and crystallizes on cooling as rhombic prisms. The acid prepared from protein substances is optically active, and its 4 per cent solution acidified with HCl has the rotation $(\alpha)_D = +25.7°$; but it is either dextrogyrate or levogyrate in a watery solution, depending upon the temperature. It forms with copper oxide a crystalline compound which is soluble in boiling-hot water and nearly insoluble in cold water, and which may be used in the preparation of the pure acid from a mixture with other bodies.

In regard to the benzoylaspartic acids and the diethylester we must refer to the work of E. FISCHER and his collaborators. For identification we make use of the analysis of the free acid and the copper salts, as well as the specific rotation.

Glutamic acid (α-aminoglutaric acid), $C_5H_9NO_4$ =  $\begin{array}{l}COOH\\ \dot{C}H(NH_2)\\ \dot{C}H_2\\ \dot{C}H_2\\ \dot{C}OOH\end{array}$, is obtained from the protein substances under the same conditions as the other monamino-acids and from the peptones (SIEGFRIED). HLASIWETZ and HABERMANN obtained 29 per cent from casein by cleavage with hydrochloric acid, while KUTSCHER could obtain only 1.8 per cent glutamic acid by cleavage with sulphuric acid. HORBACZEWSKI has obtained 15–18 per cent glutamic acid from gelatine and about the same amount from horn, while FISCHER and DÖRPINGHAUS obtained only 3 per cent from horn. FISCHER and ABDERHALDEN obtained 1.06 per cent from hæmoglobin, KUTSCHER 3.66 per cent from thymus histone, and ABDERHALDEN and PREGL[3] obtained 8 per cent from ovalbumin.

Glutamic acid crystallizes in rhombic tetrahedra or octahedra or in small leaves. It melts at 202–203° C. with partial decomposition. It dissolves in 100 parts water at 16° C., and in 1500 parts 80 per cent alcohol.

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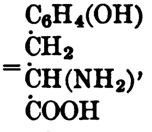
[1] Hlasiwetz and Habermann, Annal. d. Chem. u. Pharm., **159 and 169**; E. Fischer and collaborators, see foot-note 1, p. 84.

[2] Ber. d. d. chem. Gesellsch., **34**.

[3] Hlasiwetz and Habermann, l. c., **159**; Kutscher, Zeitschr. f. physiol. Chem., **28** and **38**; Horbaczewski, Maly's Jahresber., **10**; Fischer and collaborators, l. c.; Abderhalden and Pregl, Zeitschr. f. physiol. Chem., **46**.

It is insoluble in alcohol and ether. The *d*-glutamic acid obtained from proteins by boiling with an acid or from the mother-liquor from molasses is dextrorotatory, and in water has a rotation of $(\alpha)_D = +12.04°$ according to ANDRLIK.[1] Strong acids increase the rotation, and a 5 per cent solution of glutamic acid containing 9 per cent HCl has a rotation $(\alpha)_D = +31.7°$, while that obtained by heating with barium hydrate is optically inactive. The *d*-glutamic acid forms a beautifully crystalline combination with hydrochloric acid, which is nearly insoluble in concentrated hydrochloric acid. This compound is used in the isolation of glutamic acid. On boiling with cupric hydrate a beautiful crystalline copper salt, which is soluble with difficulty, is obtained. Like the monamino-acids in general, glutamic acid is not precipitated by phosphotungstic acid. In regard to the benzoylglutamic acids and the diethylester we must refer to the works of FISCHER.[2] The hydrochloride, the α-naphthylisocyanate of glutamic acid which melts at 236–237° C., the analysis of the free acid, and the specific rotation are used in its detection.

Tyrosine (*p*-oxyphenyl-α-aminopropionic acid), $C_9H_{11}NO_3$ =



$$\begin{array}{l} C_6H_4(OH) \\ \dot{C}H_2 \\ \dot{C}H(NH_2) \\ \dot{C}OOH \end{array}$$

, is produced from most protein substances (not from gelatine and reticulin) under the same conditions as leucine, which it habitually accompanies. The largest quantity of tyrosine obtained from animal proteins was obtained by FISCHER and SKITA from fibroin, namely, 10 per cent. The maximum obtained from thymus histone (KUTSCHER) was 6.3 per cent, from horn substance (R. COHN) 4.6 per cent, from casein (REACH) 4.55 per cent, from fibrin (KÜHNE) 3.86 per cent, from ovalbumin, seralbumin, and serglobulin (K. MÖRNER) 2.4, 2.0, and 3.0 per cent respectively, from syntonin (REACH) 1.37 per cent, from hæmoglobin (FISCHER and ABDERHALDEN) 1.5 per cent, and from elastin (SCHWARZ[3]) 0.34 per cent. It is especially found with leucine in large quantities in old cheese (*Τυρός*), form which it derives its name. Tyrosine has not with certainty been found in perfectly fresh organs. It occurs in the intestine in the digestion of protein substances, and it has about the same physiological and pathological importance as leucine.

Tyrosine was prepared by ERLENMEYER and LIPP from *p*-aminophenylalanine by the action of nitrous acid, and according to another method by

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[1] See Biochem. Centralbl., **3**, p. 469.

[2] l. c.

[3] Fischer and Skita, l. c.; Kutscher, Zeitschr. f. physiol. Chem., **38**; R. Cohn, *ibid.*, **26**; Reach, Virchow's Arch., **158**; Kühne, *ibid.*, **39**; K. Mörner, Zeitschr. f. physiol. Chem., **34**; Fischer and Abderhalden, *ibid.*, l. c.; Schwarz, *ibid.*, **18**.

Erlenmeyer and Halsey.[1] On fusing with caustic alkali it yields *p*-oxybenzoic acid, acetic acid, and ammonia. On putrefaction it may yield *p*-hydrocoumaric acid, oxyphenylacetic acid, and *p*-cresol.

Naturally occurring tyrosine and that obtained by the cleavage of protein substances is generally *l*-tyrosine, while that obtained by decomposition with baryta-water or prepared synthetically is inactive. v. Lippmann[2] has obtained *d*-tyrosine from beet-sprouts. The statements as to specific rotation of tyrosine are somewhat variable. For tyrosine from proteins E. Fischer has found a rotation of $(\alpha)_D = -12.56$ to 13.2° for the hydrochloric-acid solution, while Schulze and Winterstein[3] obtained higher results using tyrosine from plants, namely, $(\alpha)_D = -16.2°$. These investigators believe that when lower results are obtained a contamination with racemic tyrosine is the cause.

Tyrosine in a very impure state may be in the form of balls similar to leucine. The purified tyrosine, on the contrary, appears as colorless, silky, fine needles which are often grouped into tufts or balls. It is soluble with difficulty in water, being dissolved by 2454 parts water at 20° C. and 154 parts boiling water, separating, however, as tufts of needles on cooling. It dissolves more easily in the presence of alkalies, ammonia, or a mineral acid. It is difficultly soluble in acetic acid. Crystals of tyrosine separate from an ammoniacal solution on the spontaneous evaporation of the ammonia. One hundred parts glacial acetic acid dissolve on boiling only 0.18 parts tyrosine, and by this means, especially on adding an equal volume of alcohol before boiling, the leucine can be quantitatively separated from the tyrosine (Habermann and Ehrenfeld). The *l*-tyrosine ethyl ester crystallizes in colorless prisms which melt at 108–109° C. The naphthylisocyanate-*l*-tyrosine melts at 205–206°. Tyrosine can be oxidized with the formation of dark-colored products by various plant as well as animal oxidases, so-called tyrosinases (see Chapter I). By the enzyme occurring in beet-juice tyrosine can be converted into homogentisic acid (Gonnermann[4]). Tyrosin is identified by its crystalline form and by the following reactions:

Piria's *Test*. Tyrosine is dissolved in concentrated sulphuric acid by the aid of heat, by which tyrosine-sulphuric acid is formed; it is allowed to cool, diluted with water, neutralized by $BaCO_3$, and filtered. On the addition of a solution of ferric chloride the filtrate gives a beautiful violet color.

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[1] Erlenmeyer and Lipp, Ber. d. d. chem. Gesellsch., **15**; Erlenmeyer and Halsey, *ibid.*, **30**.

[2] *Ibid.*, **17**.

[3] See Hoppe-Seyler-Thierfelder, Handb. d. physiol. u. pathol. chem. Analyse, 7. Auflage, 1903. Also E. Fischer, Ber. d. d. chem. Gesellsch., **32**; Schulze and Winterstein, Zeitschr. f. physiol. Chem., **45**.

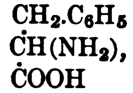
[4] Pflüger's Arch., **82**.

This reaction is disturbed by the presence of free mineral acids and by the addition of too much ferric chloride.

Hofmann's *Test.* If some water is poured on a small quantity of tyrosine in a test-tube and a few drops of Millon's reagent added and then the mixture boiled for some time, the liquid becomes a beautiful red and then yields a red precipitate. Mercuric nitrate may first be added, then, after this has boiled, nitric acid containing some nitrous acid.

Denigès' *Test,* modified by C. Mörner,[1] is performed as follows: To a few cubic centimetres of a solution consisting of 1 vol. formaline, 45 vols. water, and 55 vols. concentrated sulphuric acid add a little tyrosine in substance or in solution and heat to boiling. A beautiful permanent green coloration is obtained.

Phenylalanine (phenyl-α-aminopropionic acid), $C_9H_{11}NO_2 =$



$$C_9H_{11}NO_2 = \begin{array}{l} CH_2.C_6H_5 \\ \dot{C}H(NH_2), \\ \dot{C}OOH \end{array}$$

was first found by E. Schulze and Barbieri[2] in etiolated lupin sprouts. It is produced in the acid cleavage of protein substances. E. Fischer and his collaborators[3] obtained 3.38 per cent phenylalanine from hæmoglobin, 3.0 per cent from horn substance, 2.5 per cent from ovalbumin and casein, 1.5 per cent from fibroin, and 0.4 per cent from gelatine. Abderhalden and Schittenhelm obtained 3.89 per cent from elastin.

The *l*-phenylalanine crystallizes in small, shining leaves or fine needles which are rather difficultly soluble in cold water but readily soluble in hot water. A 5 per cent solution acidified with hydrochloric acid or sulphuric acid is precipitated by phosphotungstic acid, while a more dilute solution is not precipitated. On putrefaction, phenylalanine yields phenylacetic acid. On heating with potassium dichromate and sulphuric acid (25 per cent) an odor of phenylacetaldehyde is produced and benzoic acid is formed.

The separation and preparation of the three amino-acids, aspartic acid, glutamic acid, and tyrosine, from a mixture of hydrolytic decomposition products of protein substances is performed essentially according to the method suggested by Hlasiwetz and Habermann with the modifications and changes proposed by other investigators. The isolation and purification of the amino-acids can be best accomplished according to E. Fischer's method, which consists essentially in esterifying the acids first with hydrochloric acid and alcohol, separating the esters from their hydrochloride by means of alkali, and then fractionally distilling the esters under very low pressure, and finally saponifying the different fractions by boiling with water or by heating with baryta-water. It is not within the scope of this book to give a detailed description of these methods, therefore we must refer for further information to HOPPE-SEYLER-THIERFELDER's "Handbuch der physiologisch- und pathologisch-chemischen Analyse," 7. Auflage, and to FISCHER's [1] collected works on this subject.

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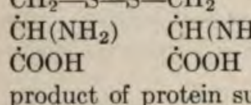
[1] Denigès, Compt. rend., **130**; C. Mörner, Zeitschr. f. physiol. Chem., **37.**

[2] Ber. d. d. chem. Gesellsch., **14,** and Zeitschr. f. physiol. Chem., **12.**

[3] See foot-note 1, p. 84.

We must here add that the preparation of the β-naphthalenesulpho-derivatives according to FISCHER and BERGELL, of the 4-nitrotoluene-2-sulpho-compounds according to SIEGFRIED, and of the α-naphthylisocyanate compounds according to NEUBERG and MANASSE [2] are also of importance in the detection and isolation of many of the amino-acids.

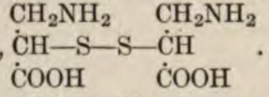
Cystine, $C_6H_{12}N_2S_2O_4$ (the disulphide of α-amino-β-thiolactic acid),



$$\begin{array}{ll} CH_2{-}S{-}S{-}CH_2 & \\ \dot{C}H(NH_2) & \dot{C}H(NH_2) \\ \dot{C}OOH & \dot{C}OOH \end{array}$$

was first obtained with positiveness as a cleavage product of protein substances by K. MÖRNER, and then also by EMBDEN. KÜLZ [3] obtained it also once as a product of tryptic digestion of fibrin. MÖRNER obtained 6.8 per cent cystine from ox-horn, 13.92 per cent from human hair, 7.62 per cent from the membrane of the hen egg, 2.53 per cent from seralbumin, 1.51 per cent from serglobulin, 1.17 per cent from fibrinogen, and 0.29 per cent from ovalbumin.

According to NEUBERG and MAYER [4] two kinds of cystine occur in nature, namely, *stone-cystine* and *protein-cystine*. Stone-cystine is the disulphide of β-amino-α-thiolactic acid,



$$\begin{array}{ll} CH_2NH_2 & CH_2NH_2 \\ \dot{C}H{-}S{-}S{-}\dot{C}H & \\ \dot{C}OOH & \dot{C}OOH \end{array}.$$

It is difficult to say which cystine we have had in the various cases where it has been found. The protein-cystine has been chiefly obtained from the protein substance, but also from calculi, while the stone-cystine has only been obtained from urinary calculi. ROTHERA could not find any difference between the stone-cystine and the cystine prepared from hair, and FISCHER and SUZUKI [5] arrived at similar results, which seems to place the existence of stone-cystine in doubt. The occurrence of two stereoisomeric cystines is not improbable, and important observations of MÖRNER show that the cystine-yielding group of the protein substances contains two cystines.

Cystine occurs in rare cases in the urine or as a calculus, and has also been found in ox-kidneys, in the liver of the horse and dolphin, and in traces in the liver of a drunkard. ABDERHALDEN [6] has found cystine in the urine and also abundantly in the organs (spleen) in a case of parental cystine diathesis.

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[1] Ber. d. d. chem. Gesellsch., **39**, p. 530. His collected works on this subject may be found in Fischer's "Untersuchungen über Aminosäuren, Polypeptide und Proteine 1899–1906," Berlin, 1906.

[2] Fischer and Bergell, Ber. d. d. chem. Gesellsch., **35**; Neuberg and Manasse, *ibid.*, **38**; Siegfried, Zeitschr. f. physiol. Chem., **43**.

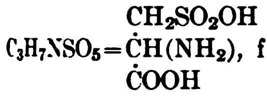
K. Mörner, *ibid.*, **28**, **34**, and **42**; Embden, *ibid.*, **32**; Külz, Zeitschr. f. Biologie, **27**.

[4] Zeitschr. f. physiol. Chem., **44**.

[5] Rothera, Journ. of Physiol., **32**; Fischer and Suzuki, Zeitschr. f. physiol. Chem., **45**.

[6] Zeitschr. f. physiol. Chem., **38**.

The constitution of cystine has been explained by Friedmann,[1] and he has also established the relationship between cystine and taurine. Cystine is the disulphide of cysteine, which is α-amino-β-thiolactic acid. From cysteine Friedmann obtained cysteinic acid (aminosulphopropionic acid),



$$C_3H_7NSO_5 = \begin{array}{l} CH_2SO_2OH \\ \dot{C}H(NH_2) \\ \dot{C}OOH \end{array}$$

from which taurine is produced by splitting off CO_2.

Cystine has also been prepared synthetically. Starting from ethyl formyl hippurate, Erlenmeyer, Jr., and Stoop first prepared the benzoylserine ester, and then with phosphorus pentasulphide they obtained the benzoylcystine ester. On splitting the latter with HCl they obtained cysteine, and then inactive cystine on oxidation. Gabriel[2] has also prepared an isocysteine by the cleavage of rhodandihydrouracil with hydrochloric acid, and then inactive cystine by the oxidation of this isocysteine.

Cystine crystallizes in thin, colorless, hexagonal plates. It is not soluble in water, alcohol, ether, or acetic acid, but dissolves in mineral acids and oxalic acid. It is also soluble in alkalies and ammonia, but not in ammonium carbonate. Cystine is optically active, being levorotatory. Mörner found it to be $(\alpha)_D = -224.3°$. On heating with hydrochloric acid it can, according to Mörner, be changed into a modification crystallizing in needles and with a weaker levorotatory power, and indeed it can be changed into a dextrorotatory modification. On heating with HCl to 165° for 12–15 hours Neuberg and Mayer obtained inactive cystine. It is questionable whether this is identical with the inactive cystine prepared by Erlenmeyer synthetically. By fungus fermentation with Aspergillus niger they obtained dextrorotatory cystine. Cystine has no melting-point but slowly decomposes at 258–261°. On boiling cystine with caustic alkali it decomposes and yields alkali sulphide, which can be detected by lead acetate or sodium nitroprusside. According to Mörner 75 per cent of the total sulphur is separated. On treatment of cystine with tin and hydrochloric acid it develops only a little sulphuretted hydrogen, and is converted into cysteine. On shaking a solution of cystine in an excess of sodium hydrate with benzoyl chloride, a voluminous precipitate of benzoyl cystine is obtained (Baumann and Goldmann[3]). The benzoyl compound melts at 182–184°. The phenylcyanate compound melts at 160° and on boiling with 25 per cent HCl is transformed into its anhydride, a hydantoin melting at 119°. Cystine forms crystalline salts with mineral acids and with bases. For isolating and separating cystine the precipitation with mercuric acetate is especially suited. On heating upon platinum-foil cystine does not melt, but ignites and burns with a bluish-green flame, with the generation of a peculiar sharp odor. When warmed with nitric acid it dissolves with decomposition and leaves on evaporation a reddish-brown residue, which does not give the murexid test. Cystine is gradually precipitated from its sulphuric acid solution by phosphotungstic acid.

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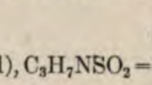
[1] Hofmeister's Beiträge, **3**, p. 1.

[2] Erlenmeyer and Stoop, Ber. d. d. chem. Gesellsch., **36**; Gabriel, *ibid.*, **38**.

[3] Mörner, Zeitschr. f. physiol. Chem., **34**; Baumann and Goldmann, *ibid.*, **12**.

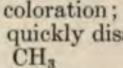
Stone-cystine, according to NEUBERG and MAYER, differs in many regards from the ordinary cystine, among which the following will be mentioned: The optically active stone-cystine crystallizes in needles, the specific rotation is $(\alpha)_D = -206°$; it melts at 190–192° with marked swelling up. The benzoyl compound melts at 157–159°; the phenylcyanate compound melts at 170–172°, and it is not changed on boiling with hydrochloric acid.

In the detection and identification of cystine we make use of the crystalline form, the behavior on heating on platinum-foil, and the sulphur reaction after boiling with alkali. As to its preparation from protein substances see K. MÖRNER.[1] In regard to the detection of cystine in the urine see Chapter XV.

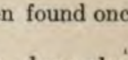


Cysteine (α-amino-β-thiolactic acid), $C_3H_7NSO_2 = \begin{array}{l} CH_2.SH \\ \dot{C}H(NH_2) \\ \dot{C}OOH \end{array}$, is formed from cystine by reduction with tin and hydrochloric acid. It is also produced in the cleavage of protein substances, but this is considered by MÖRNER and PATTEN[2] as a secondary formation, while EMBDEN considers it as primary from proteins poor in sulphur. Besides α-amino-β-thiocysteine occurring in the proteins we may probably also have a β-amino-α-thiocysteine. According to MÖRNER the thiolactic acid which he obtained on the decomposition of cystine probably originates from the latter, while the α-amino-β-thiocysteine is probably the mother-substance of the alanine obtained at the same time. Cysteine can be readily converted into cystine.

Towards alkalies and lead acetate it acts like cystine. With sodium nitroprusside and alkali it gives a deep purple-red coloration; with ferric chloride the solution gives an indigo-blue coloration which quickly disappears.



Thiolactic acid (α-thiolactic acid), $C_3H_6SO_2 = \begin{array}{l} CH_3 \\ \dot{C}H.SH \\ \dot{C}OOH \end{array}$, has been found once as a cleavage product of ox-horn by BAUMANN and SUTER. It has been shown by FRIEDMANN that this acid is a regular cleavage product of keratin substances, and that it can also be obtained from the proteins. FRÄNKEL[3] obtained the acid from hæmoglobin. The pyroracemic acid obtained by MÖRNER as a decomposition product from several protein substances originates, according to MÖRNER, only in part from the cystine.



Taurine[4] (aminoethylsulphonic acid), $C_2H_7NSO_3 = \begin{array}{l} CH_2.NH_2 \\ \dot{C}H_2.SO_2.OH \end{array}$, has not been obtained as a cleavage product of protein substances; still its origin from proteins has been shown by Friedmann by the close relationship that taurine bears to cysteine. Taurine is especially known as a cleavage product of taurocholic acid and may occur to a slight extent in the intestinal contents. Taurine has also been found in the lungs and kidneys of oxen and in the blood and muscles of cold-blooded animals.

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[1] Zeitschr. f. physiol. Chem., **34**.

[2] See foot-note 2, p. 28.

[3] Suter, Zeitschr. f. physiol. Chem., **20**; Friedmann, Hofmeister's Beiträge, **3**; Fränkel, Sitzungsber. d. Wien. Akad., **112**, II, *b*, 1903.

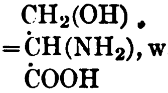
[4] Taurine does not belong to the cleavage products of the proteins, but for practical reasons it will be described in connection with cystine.

Taurine crystallizes in colorless, often in large, shining, 4- or 6-sided prisms. It dissolves in 15–16 parts of water at ordinary temperatures, but rather more easily in warm water. It is insoluble in absolute alcohol and ether; in cold spirits of wine it dissolves slightly, but better when warm. Taurine yields acetic and sulphurous acids, but no alkali sulphides, on boiling with strong caustic alkali. The content of sulphur can be determined as sulphuric acid after fusing with saltpetre and soda. Taurine combines with metallic oxides. The combination with mercuric oxide is white, insoluble, and is formed when a solution of taurine is boiled with freshly precipitated mercuric oxide (J. Lang [1]). This compound may be used in detecting the presence of taurine. Taurine is not precipitated by metallic salts.

The preparation of taurine from ox-bile is very simple. The bile is boiled a few hours with hydrochloric acid. The filtrate from the dyslysin and choloidic acid is concentrated well on the water-bath, and filtered hot so as to remove the common salt and other substances which have separated. The solution is evaporated to dryness and the residue dissolved in 5 per cent hydrochloric acid, and precipitated with 10 vols. 95 per cent alcohol. The crystals are readily purified by recrystallization from water. The alcoholic solution can be used for the preparation of glycocoll. After the evaporation of the alcohol, the residue is dissolved in water, treated with a solution of lead hydroxide, filtered, the lead removed by H_2S, and the filtrate strongly concentrated. The crystals which separate are dissolved and decolorized by animal charcoal and the solution then evaporated to crystallization.

Though taurine shows no positive reactions, it is chiefly identified by its crystalline form, by its solubility in water and insolubility in alcohol, by its combination with mercuric oxide, by its non-precipitability by metallic salts, and above all by its sulphur content.

Oxymonamino-acids.

Serine (α-amino-β-oxypropionic acid), $C_3H_7NO_3 =$  $\begin{array}{l}CH_2(OH)\\ \dot{C}H(NH_2)\\ \dot{C}OOH\end{array}$, was obtained by E. Fischer and his collaborators [2] as a cleavage product from fibroin (1.6 per cent), horn substance (0.68 per cent), sericine, gelatine (0.4 per cent), and casein. Kossel and Dakin [3] obtained 7.8 per cent from salmine. Synthetically it was prepared by E. Fischer and Leuchs [4] from ammonia.

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[1] See Maly's Jahresber., 6.

[2] See foot-note 1, p. 84.

[3] Zeitschr. f. physiol. Chem., 41.

[4] Ber. d. d. chem. Gesellsch., 35, and Sitzungsber. d. Akad. d. Wiss., Berlin, 1902.

hydrocyanic acid, and glycolyl aldehyde. Serine has also been prepared by ERLENMEYER, JR., and STOOP[1] by starting with ethyl formyl hippurate and converting it by reduction into benzoylserine ester, from which benzoylserine was obtained by saponification with alcoholic potash, and then from this, serine was obtained by boiling with sulphuric acid.

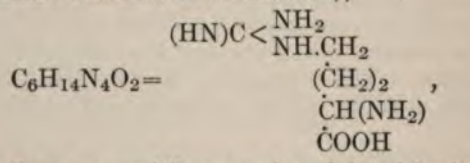
Isoserine (β-amino-α-oxypropionic acid) has been prepared by ELLINGER from diaminopropionic hydrobromide and silver nitrite, and by NEUBERG and SILBERMANN[2] from diaminopropionic hydrochloride.

Serine does not dissolve readily in cold water (23 parts water at 20° C.), but more easily in hot water. The solution is inactive and has a sweet taste. Serine crystallizes from water in thin plates, which melt at 240° with the generation of a gas.

According to SKRAUP, oxyaminosuccinic acid, $C_{11}H_7NO_5$, is very probably a hydrolytic cleavage product of the proteins. This acid has been prepared synthetically by NEUBERG and SILBERMANN from diaminosuccinic acid and barium nitrite in sulphuric-acid solution. Oxyaminosuberic acid, $C_8H_{15}NO_5$, has been found by WOHLGEMUTH as a cleavage product of a liver nucleoproteid, and the acid $C_6H_{13}NO_6$, isolated by ORGLER and NEUBERG[3] from chondroitin-sulphuric acid, but not from protein, and considered by them as tetraoxyaminocaproic acid, seems also to belong to the oxyamino-acid group.

2. Diamino-acids (Hexone Bases).

Arginine (guanidine-α-aminovalerianic acid),



$$C_6H_{14}N_4O_2 = \begin{array}{l} (HN)C<\begin{smallmatrix}NH_2\\ NH.CH_2\end{smallmatrix} \\ \qquad\quad\ (\dot{C}H_2)_2 \\ \qquad\quad\ \dot{C}H(NH_2) \\ \qquad\quad\ \dot{C}OOH \end{array},$$

first discovered by SCHULZE and STEIGER in etiolated lupin- and pumpkin-sprouts, has later been found in other germinating plants, in tubers and roots. GULEWITSCH has found arginine in the ox-spleen. It was first found by HEDIN as a cleavage product of horn substance, gelatine, and several proteins, and then by KOSSEL and his pupils as a general cleavage product of protein substances as a class. The greatest quantity was obtained from the protamines; but the histones and certain plant proteins (edestin and the protein from pine seeds) also yield abundant arginine. Arginine also occurs among the products of tryptic digestion (KOSSEL and KUTSCHER).

On boiling with baryta-water as well as by the action of an enzyme,

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[1] Ber. d. d. chem. Gesellsch., **35**.

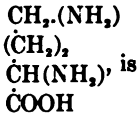
[2] Ellinger, *ibid.*, **37**; Neuberg and Silbermann, *ibid.*, **37**.

[3] Skraup, Zeitschr. f. physiol. Chem., **42**; Neuberg and Silbermann, *ibid.*, **44**; Wohlgemuth, *ibid.*, **44**; Orgler and Neuberg, *ibid.*, **37**.

arginase, discovered by **Kossel** and **Dakin**,[1] arginine yields urea and ornithine. Arginine has been prepared synthetically from ornithine (α-δ-diaminovalerianic acid) and cyanamide by **Schulze** and **Winterstein**.[2]

Arginine crystallizes in rosette-like tufts, plates, or thin prisms, is readily soluble in water and nearly insoluble in alcohol. With several acids and metallic salts it forms crystalline salts and double salts respectively. Its acidified watery solution is precipitated by phosphotungstic acid. The most important salts are the copper-nitrate $(C_6H_{14}N_4O_2)_2.Cu(NO_3)_2+3H_2O$ and the silver salts $C_6H_{14}N_4O_2.HNO_3+AgNO_3$ (the more readily soluble) and $C_6H_{14}N_4O_2.AgNO_3+\frac{1}{2}H_2O$ (the more difficultly soluble) and its compound with picrolonic acid (**Steudel**[3]).

Arginine is dextrorotatory, but the arginine obtained by **Kutscher** in the tryptic digestion of fibrin was inactive. On oxidation with permanganate it splits off guanidine, which can be precipitated with sodium picrate. **Orglmeister**[4] bases his method for the quantitative estimation of arginine in mixtures obtained by hydrolysis upon this fact.

Ornithine (α-δ-diaminovalerianic acid), $C_5H_{12}N_2O_2 =$  $\begin{array}{l} CH_2.(NH_2) \\ \dot{(C}H_2)_2 \\ \dot{C}H(NH_2) \\ \dot{C}OOH \end{array}$, is not a primary cleavage product of proteins, but is formed from arginine on boiling with baryta-water. **Jaffe**,[5] who first discovered this body, obtained it as a cleavage product from ornithuric acid, which is found in the urine of hens fed with benzoic acid. The ornithine which E. **Fischer** and recently **Sörensen**[6] have prepared synthetically yields, as shown by **Ellinger**, putrescine (tetramethylenediamine), $C_4H_8(NH_2)_2$, on putrefaction. A. **Loewy** and **Neuberg**[7] have shown that ornithine is split into putrescine and CO_2 in the organism of cystinuria patients.

Ornithine is a non-crystalline substance which dissolves in water, giving an alkaline reaction, and yields several crystalline salts. It is precipitated by phosphotungstic acid and several metallic salts, but not by silver nitrate and baryta-water (differing from arginine). Ornithine hydrochloride is dextrorotatory; the synthetically prepared is inactive. On shaking ornithine with benzoyl chloride and caustic soda it is converted into dibenzoylornithine (ornithuric acid). On splitting artificially prepared racemic ornithuric acid **Sörensen** has shown that the naturally occurring ornithuric acid is identical with the dextrorotatory α-δ-dibenzoyldiaminovalerianic acid.

Diaminoacetic acid, $C_2H_6N_2O_2 = CH(NH_2)_2COOH$, was obtained by **Drechsel**[8] as a cleavage product of casein by boiling with tin and hydrochloric acid. It crystallizes in prisms and gives a monobenzoyl compound which is not very soluble in cold water and is nearly insoluble in alcohol, and can be used in the isolation of the acid.

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[1] Schulze and Steiger, Zeitschr. f. physiol. Chem., 11; Schulze and Castoro, *ibid.*, 41; Gulewitsch, *ibid.*, 30; Hedin, *ibid.*, 20 and 21; Kossel and Kutscher, *ibid.*, 22, 25, 26; Kossel and Dakin, *ibid.*, 41.

[2] Ber. d. d. chem. Gesellsch., 32, and Zeitschr. f. physiol. Chem., 34.

[3] Zeitschr. f. physiol. Chem., 37 and 44.

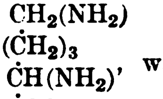
[4] Hofmeister's Beiträge, 7.

[5] Ber. d. d. chem. Gesellsch., 10 and 11.

[6] Fischer, *ibid.*, 34; Sörensen, Zeitschr. f. physiol. Chem., 44.

[7] Ellinger, Zeitschr. f. physiol. Chem., 29; Loewy and Neuberg, *ibid.*, 43.

[8] Ber. d. sächs. Ges. d. Wissensch., 44.



Lysine (α-ε-diaminocaproic acid), $C_6H_{14}N_2O_2 = \begin{array}{l} CH_2(NH_2) \\ (\dot{C}H_2)_3 \\ \dot{C}H(NH_2) \\ \dot{C}OOH \end{array}$, was first obtained by DRECHSEL as a cleavage product of casein. Later he and his pupils, as well as KOSSEL and others, found it among the cleavage products of various proteins. It has not been detected in some vegetable proteins such as zein and gluten-protein. E. SCHULZE found lysine in germinating plants of the Lupinus luteus, and WINTERSTEIN found it in ripe cheese. It has been obtained in largest amounts (28.8 per cent) by KOSSEL and DAKIN[1] from the protamine α-cyprinine.

Lysine has been synthetically prepared by E. FISCHER and WEIGERT.[2] This lysine was inactive, while that prepared from protein is always optically active and dextrorotatory. On heating with barium hydrate it is converted into the inactive modification. According to ELLINGER[3] lysine yields cadaverine (pentamethylenediamine), $C_5H_{10}(NH_2)_2$, on putrefaction, and this base is formed from the lysine in the organism of those with cystinuria and at the same time CO_2 is split off (A. LOEWY and NEUBERG).

Lysine is readily soluble in water but is not crystalline. The aqueous solution is precipitated by phosphotungstic acid but not by silver nitrate and baryta-water (differing from arginine and histidine). It gives two hydrochlorides with hydrochloric acid, and with platinum chloride a chloroplatinate which is precipitable by alcohol and has the composition $C_6H_{14}N_2O_2.H_2PtCl_6+C_2H_5OH$. It gives two silver salts with $AgNO_3$; one has the formula $AgNO_3+C_6H_{14}N_2O_2$ and the other $AgNO_3+C_6H_{14}N_2O_2.HNO_3$. With benzoyl chloride and alkali, lysine forms an acid, *lysuric acid*, $C_6H_{12}(C_7H_5O)_2N_2O_2$ (DRECHSEL), which is homologous with ornithuric acid and whose difficultly soluble acid barium salt may be used in the separation of lysine.[4] The rather insoluble picrate, which is precipitated from a not too dilute solution of the hydrochloride by sodium picrate, may also be used in the detection of lysine.

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[1] Drechsel, Arch. f. (Anat. u.) Physiol., 1891, and Ber. d. d. chem. Gesellsch., 25; Siegfried, Arch. f. (Anat. u.) Physiol., 1891, and Ber. d. d. chem. Gesellsch., 24; Hedin, Zeitschr. f. physiol. Chem., 21; Kossel, *ibid.*, 25; Kossel and Mathews, *ibid.*, 25; Kossel and Kutscher, *ibid.*, 31; Kutscher, *ibid.*, 29; Schulze, *ibid.*, 28; Winterstein, cited in Schulze and Winterstein, Ergebnisse der Physiologie, I, Abt. 1, 1902; Kossel and Dakin, Zeitschr. f. physiol. Chem., 40.

[2] Ber. d. d. chem. Gesellsch., 35.

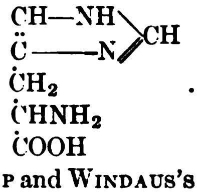
[3] Zeitschr. f. physiol. Chem., 29.

[4] Drechsel, Ber. d. d. chem. Gesellsch., 28; see also C. Willdenow, Zeitschr. f. physiol. Chem., 25.

Kutscher and **Lohmann**[1] have found a lysine having somewhat different properties in the final products of pancreas autolysis.

Lysatine or Lysatinine. The formula of this substance is either $C_6H_{13}N_3O_2$ or $C_6H_{11}N_3O+H_2O$. In the first case this base would appear to be a homologue of creatine, $C_4H_9N_3O_2$, and in the other case a homologue of creatinine, $C_4H_7N_3O$, and this is the reason why this body is called lysatine as well as lysatinine. It is still a question whether lysatine is a chemical individual or, as **Hedin** suggests, only a mixture of lysine and arginine.[2]

Histidine, $C_6H_9N_3O_2$, is perhaps not a diamino-acid, as **Fränkel**[3] first showed, but, according to the investigations of H. **Pauly**, **Knoop** and **Windaus**,[4] is an α(?)-amino-β-imidazolpropionic acid,



$$\begin{array}{l} \left.\begin{array}{l} \mathrm{CH{-}NH} \\ \ddot{\mathrm{C}}\text{——}\mathrm{N} \end{array}\right\rangle \mathrm{CH} \\ \dot{\mathrm{C}}\mathrm{H_2} \\ \dot{\mathrm{C}}\mathrm{HNH_2} \\ \dot{\mathrm{C}}\mathrm{OOH} \end{array}$$

.

Fränkel[5] has made several objections to **Pauly**, **Knoop** and **Windaus's** view that histidine is an imidazol derivate, which seem to be well founded, therefore the question as to the constitution of histidine remains still an open one.

Histidine[6] was first discovered by **Kossel** in the cleavage products of sturine. It was then found by **Hedin** in the cleavage products of proteins by acid hydrolysis, and by **Kutscher** among the products of tryptic digestion, and finally also as a cleavage product of different protein substances. It does not occur in the protamines, with the exception of sturine. Of the protein bodies globin (from horse-hæmoglobin) seems to be richest in histidine, as **Abderhalden** found 10.96 per cent. It also occurs in germinating plants (E. **Schulze**[7]).

Histidine crystallizes in colorless needles and plates and is readily soluble in water, but less soluble in alcohol, and has an alkaline reaction. It is precipitated by phosphotungstic acid, but this precipitate is soluble in an excess of the precipitant (**Fränkel**). With silver nitrate alone the aqueous solution is not precipitated; on the careful addition of ammonia or baryta-water an amorphous precipitate, which is readily soluble in an excess of ammonia, is obtained. Histidine can be precipitated by mercuric chloride, or, still better, by the sulphate acidified with sulphuric acid, and can in this way be separated from the diamino-acids as well as from the monamino-acids (Kossel and Patten). The hydrochloride crystallizes in beautiful plates (Bauer), dissolves rather readily in water, but is insoluble in alcohol and ether. With hydrochloric acid and methyl alcohol it gives the dihydrochloride of histidine methyl ester, which melts at 196°. Histidine is levorotatory, while its solution in hydrochloric acid is dextrorotatory. On warming it gives the biuret test (Herzog[1]), and it also gives Weidel's reaction if performed as suggested by Fischer (see Xanthine, Chapter V) (Fränkel). It gives a very beautiful diazo-reaction with diazobenzenesulphonic acid in solutions made alkaline with sodium carbonate, which according to Pauly is deep cherry-red in dilutions of 1:20 000 and still markedly red in 1:100 000 (tyrosine gives a similar reaction).

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[1] Zeitschr. f. physiol. Chem., 41.

[2] Hedin, *ibid.*, 21; Siegfried, *ibid.*, 35.

[3] Sitzungsber. d. Wien. Akad., 112, II, *b*, 1903.

[4] Pauly, Zeitschr. f. physiol. Chem., 42; Knoop and Windaus, Hofmeister's Beiträge, 7.

[5] Hofmeister's Beiträge, 8.

[6] As histidine is always obtained with the diamino-acids it is called a hexone base, hence it will be treated here with the diamino-acids.

[7] Kossel, Zeitschr. f. physiol. Chem., 22; Hedin, *ibid.*, Kutscher, *ibid.*, 25; Wetzel, *ibid.*, 26; Lawrow, *ibid.*, 28, and Ber. d. d. chem. Gesellsch., 34; Kossel and Kutscher, Zeitschr. f. physiol. Chem., 31; Hart, *ibid.*, 33; Abderhalden, *ibid.*, 37; Schulze, *ibid.*, 24 and 28.

In the preparation of the above bases we can first precipitate all the bases by phosphotungstic acid, when the monamino-acids remain in solution. The precipitate is decomposed in boiling water by barium hydrate and the bases obtained as silver compounds from this filtrate. In regard to further details we must refer to the works of Drechsel and Hedin already cited. Kossel and Kutscher and recently Winterstein[2] have suggested a method of separating histidine and arginine as silver compounds from lysine, and Kossel and Patten have proposed a method of separating histidine from arginine by means of mercuric sulphate.

We give below a tabulation of the amounts of the three hexone bases found in certain protein substances (in weight per cent):

	Arginine	Lysine	Histidine
Sturine[3]	58.2	12.0	12.9
Cyprinine (α)[7]	4.9	28.8	0.0
Other protamines[3]	62.5—87.4	0.0	0.0
Histones[3]	14.36—15.52	7.7—8.3	1.21—2.34
Casein[4]	4.70—4.84	1.92—5.80	2.53—2.59
Syntonin (from meat)[4]	5.06	3.26	2.66
Heterosyntonose[4]	8.53	3.08—7.03	0.37—1.12
Protosyntonose[4]	4.55	3.08	3.35
Edestin[5]	11.0—14.07	1.3	1.17
Proteid from coniferæ seeds[5]	10.9—11.3	0.25—0.79	0.62—0.78
Gluten casein[3]	4.4	2.15	1.16
Gluten proteins[3]	2.75—3.13	0.0	0.43—1.53
Gelatine[3] and [4]	7.62—9.3	2.49—6.0	0.40
Elastin[6]	0.3	+	0.027

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[1] Kossel and Patten, Zeitschr. f. physiol. Chem., **38**; Bauer, *ibid.*, **22**; Herzog, *ibid.*, **37**.

[2] Kossel and Kutscher, *ibid.*, **31**; Winterstein, *ibid.*, **45**; Kossel and Patten, l. c.

[3] Kossel and Kutscher, Zeitschr. f. physiol. Chem., **31**.

[4] Hart, *ibid.*, **33**.

[5] Schulze and Winterstein, *ibid.*, **33**; see also Kossel, Ber. d. d. chem. Gesellsch., **34**, 3236.

[6] Kossel and Kutscher, Zeitschr. f. physiol. Chem., **25**, and Richards and Gies, Amer. Journ. of Physiol., **7**.

[7] Kossel and Dakin, Zeitschr. f. physiol. Chem., **40**.

Oxydiamino-acids.

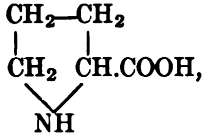
Oxydiaminosebacic acid, $C_{10}H_{20}N_2O_5$, has been isolated as a copper salt by Wohlgemuth [1] from a nucleoproteid of the liver. The free acid was obtained as small white plates. It is soluble with difficulty in hot water, insoluble in cold water and in alcohol. It was optically inactive in hydrochloric acid. The beautifully crystalline phenylcyanate compound had a melting-point of 206°.

Dioxydiaminosuberic acid, $C_8H_{16}N_2O_6$, has been obtained by Skraup [2] on the hydrolysis of casein with hydrochloric acid. The copper salt crystallizes in beautiful deep bluish-violet rosettes which are composed of long, irregular, right-angled plates. It is quite soluble in cold water. The free acid crystallizes in fern-like formations. Besides this acid Skraup obtained two other acids which he calls *caseanic acid*, $C_9H_{16}N_2O_7$, and *caseinic acid*, $C_{12}H_{24}N_2O_5$. The caseanic acid crystallizes, melts at 190–191°, is tribasic, and is probably an oxydiamino-acid. The caseinic acid is dibasic and occurs in two modifications. The one which melts at 228° is faintly dextrorotatory; the other modification melts at 245° and is optically inactive. Both crystallize, but the inactive form does not yield well-defined crystals. Caseinic acid seems also to be an oxydiamino-acid.

Diaminotrioxydodecanoic acid, $C_{12}H_{26}N_2O_5$, is an acid obtained by Fischer and Abderhalden [3] on the hydrolysis of casein and seems to stand close to Skraup's caseinic acid, but differs from it in its optical properties. This acid is faintly levorotatory: $(\alpha)_D = \text{about} -9°$. It crystallizes in plates, which grow into rosettes or spherical aggregations. It has a faint bitter taste, gives a crystalline hydrochloride which is slightly soluble in strong hydrochloric acid, and gives a crystalline copper salt.

3. Pyrrol and Indol Derivatives.

α-Pyrrolidine-carboxylic acid, abbreviated to **α-Proline**, $C_5H_9NO_2$,



was prepared by E. Fischer as a cleavage product from casein (3.2 per cent) and ovalbumin (1.55 per cent), and by him and his collaborators in the tryptic digestion of casein, and as a cleavage product of hæmoglobin (1.46 per cent), gelatine (5.2 per cent), horn substance (3.60 per cent), and from silk fibroin.[4] The acid thus obtained was generally the levorotatory modification. Kossel and Dakin [5] obtained 11 per cent α-proline from salmine, while Abderhalden and his co-workers[6] obtained 2.25 per cent from ovalbumin, 1.46 from thymus histone, 1.74 from elastin, and 3.4–3.5 per cent from keratin substances. α-Proline also occurs in scombrine and clupeine, but not in sturine, which according to KOSSEL seems to contradict the view as to the common origin of ornithine and α-proline.

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[1] Ber. d. d. chem. Gesellsch., 37, and Zeitschr. f. physiol. Chem., 44.

[2] Zeitschr. f. physiol. Chem., 42.

[3] *Ibid.*

[4] Fischer, *ibid.*, 33 and 35. See also foot-note 1, p. 84.

[5] Zeitschr. f. physiol. Chem., 41.

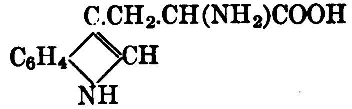
[6] Abderhalden and Pregl, *ibid.*, 46; with Rona, *ibid.*, 41; with Schittenhelm, *ibid.*, 41; with Wells and Le Count, *ibid.*, 46.

SÖRENSEN[1] by means of a general method of preparing α-amino-acids synthetically has prepared α-amino-δ-oxyvalerianic acid from phthalimide-malonic ester and has obtained α-proline from this by evaporating with hydrochloric acid, at the same time splitting off water.

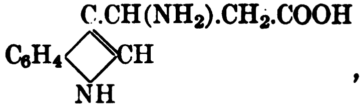
This acid is readily soluble in water and alcohol and crystallizes in flat needles which melt at 203–206° C. with an odor of pyrrolidine. The solution acidified with sulphuric acid is precipitated by phosphotungstic acid. In the detection of this acid we make use of the copper salt, the anhydride of the phenylisocyanate compound (melting-point 144°), and the picrate (ALEXANDROFF[2]). The inactive acid and its compounds show somewhat varying properties. In regard to the detection of this acid we refer to p. 91.

In the hydrolysis of gelatine and casein E. FISCHER[3] obtained an amino-acid having the formula $C_5H_9NO_3$, which on reduction yielded α-pyrrolidine-carboxylic acid, and which according to FISCHER is an oxypyrrolidine-α-carboxylic acid. LEUCHS[4] has synthetically prepared two similar, inactive acids.

Indolaminopropionic acid (tryptophane, proteinochromogen), $C_{11}H_{12}N_2O_2$,



or



is one of the cleavage products of the proteins formed in tryptic digestion and other deep decompositions of the proteins, such as putrefaction, cleavage with baryta-water or sulphuric acid. It gives a reddish-violet product with chlorine or bromine which is called *proteinochrome*. NENCKI[5] considered tryptophane, which name is generally given to this acid, as the mother-substance of various animal pigments.

Tryptophane was first prepared in a pure form by HOPKINS and COLE,[6] and they considered it as skatolaminoacetic acid. After ELLINGER[7] showed that skatolcarbonic acid (SALKOWSKI) and skatolacetic acid (NENCKI) were indolacetic acid and indolpropionic acid respectively, we have considered tryptophane as indolaminopropionic acid.

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[1] Zeitschr. f. physiol. Chem., **44**.

[2] In regard to the preparation of the phenylisocyanate compounds of the amino-acids, see Paal, Ber. d. d. chem. Gesellsch., **27**; Mouneyrat, *ibid.*, **33**, and Hoppe-Seyler-Thierfelder's Handbuch, 7. Aufl.; Alexandroff, Zeitschr. f. physiol. Chem., **46**.

[3] Ber. d. d. chem. Gesellsch., **35** and **36**.

[4] *Ibid.*, **38**.

[5] In regard to trytophane, see Stadelmann, Zeitschr. f. Biologie, **26**; Neumeister, *ibid.*, **26**; Nencki, Ber. d. d. chem. Gesellsch., **28**; Beitler, *ibid.*, **31**; Kurajeff, Zeitschr. f. physiol. Chem., **26**; Klug, Pflüger's Arch., **86**.

[6] Journ. of Physiol., **27**.

[7] Ber. d. d. chem. Gesellsch., **37** and **38**.

Tryptophane crystallizes in shining plates which are readily soluble in hot water, less soluble in cold water and in alcohol. On heating sufficiently, it yields indol and skatol. It gives the ADAMKIEWICZ-HOPKINS reaction and a rose-red coloration on the addition of bromine-water (tryptophane reaction). If a pine stick moistened with hydrochloric acid and then washed off be introduced into a concentrated tryptophane solution, it becomes purple-colored on drying (pyrrol reaction). Tryptophane, as HOPKINS and COLE[1] showed later, yields skatolacetic acid (indolpropionic acid) on anaerobic putrefaction, and skatolcarbonic acid (indolacetic acid), skatol, and indol on aerobic putrefaction.

In regard to the somewhat complicated method of preparation we must refer to the original work of HOPKINS and COLE.

Skatosine, $C_{10}H_{16}N_2O_2$, is a base first obtained by BAUM in the pancreas autodigestion and later studied by SWAIN. It develops an indol- or skatol-like odor on fusing with potassium hydrate. LANGSTEIN[2] obtained a substance, which is perhaps identical with skatosine, in the very lengthy peptic digestion of blood proteid.

The putrefactive products of the proteins will be in part treated in Chapter IX (intestinal putrefaction) and in part in Chapter XV (putrefactive products in the urine).

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[1] Journ. of Physiol., **29**; see also Ellinger and Gentzen, Hofmeister's Beiträge, **4**.

[2] Baum, Hofmeister's Beiträge, **3**; Swain, *ibid.*; Langstein, see Hofmeister, Über Bau und Gruppierung der Eiweisskörper, in Ergebnisse der Physiologie, I, Abt. 1, 1902.

CHAPTER III.

THE CARBOHYDRATES

We designate by this name bodies which are especially abundant in the plant kingdom. As the protein bodies form the chief portion of the solids in animal tissues, so the carbohydrates form the chief portion of the dry substance of the plant structure. They occur in the animal kingdom only in proportionately small quantities, either free or in combinations with more complex molecules, forming compound proteids. Carbohydrates are of extraordinarily great importance as food for both man and animals.

The carbohydrates contain only *carbon*, *hydrogen*, and *oxygen*. The last two elements occur, as a rule, in the same proportion as they do in water, namely, 2:1, and this is the reason why the name carbohydrates has been given to them. This name is not quite pertinent, if strictly considered; because we not only have bodies, such as acetic acid and lactic acid, which are not carbohydrates and still have their oxygen and hydrogen in the same proportion as in water, but we also have a sugar (rhamnose, $C_6H_{12}O_5$) which has these two elements in another proportion. At one time it was thought possible to characterize as carbohydrates those bodies which contained 6 atoms of carbon, or a multiple, in the molecule, but this is not considered tenable at the present time. We have true carbohydrates containing less than 6, and also those containing 7, 8, and 9 carbon atoms in the molecule. The carbohydrates have no properties or characteristics in general which differentiate them from other bodies; on the contrary, the various carbohydrates are in many cases very different in their external properties. Under these circumstances it is very difficult to give a positive definition for the carbohydrates.

From a chemical standpoint we can say that all carbohydrates are aldehyde or ketone derivatives of polyhydric alcohols. The simplest carbohydrates, the simple sugars or monosaccharides, are either aldehyde or ketone derivatives of such alcohols, and the more complex carbohydrates seem to be derived from these by the formation of anhydrides. It is a fact that the more complex carbohydrates yield two or even more molecules of the simple sugars when made to undergo hydrolytic splitting.

The carbohydrates are generally divided into three chief groups, namely, *monosaccharides*, *disaccharides*, and *polysaccharides*.

Our knowledge of the carbohydrates and their structural relationships has been very much extended by the pioneering investigations of KILIANI[1] and especially those of E. FISCHER.[2]

As the carbohydrates occur chiefly in the plant kingdom it is naturally not the place here to give a complete discussion of the numerous carbohydrates known up to the present time. According to the plan of this work it is only possible to give a short review of those carbohydrates which occur in the animal kingdom or are of special importance as food for man and animals.

Monosaccharides.

All varieties of sugars, the monosaccharides as well as disaccharides, are characterized by the termination "ose," to which a root is added signifying their origin or other relations. According to the number of carbon atoms, or more correctly oxygen atoms, contained in the molecule the monosaccharides are divided into *trioses*, *tetroses*, *pentoses*, *hexoses*, *heptoses*, and so on.

All monosaccharides are either aldehydes or ketones of polyhydric alcohols. The former are termed *aldoses* and the latter *ketoses*. Ordinary dextrose is an aldose, while ordinary fruit sugar (levulose) is a ketose. The difference may be shown by the structural formulæ of these two varieties of sugar:

$$\text{Dextrose}=CH_2(OH).CH(OH).CH(OH).CH(OH).CH(OH).CHO;$$
$$\text{Levulose}=CH_2(OH).CH(OH).CH(OH).CH(OH).CO.CH_2(OH).$$

A difference is also observed on oxidation. The aldoses can be converted into oxyacids having the same quantity of carbon, while the ketoses yield acids having less carbon. On mild oxidation the aldoses yield monobasic oxyacids and dibasic acids on more energetic oxidation. Thus ordinary dextrose yields gluconic acid in the first case and saccharic acid in the second.

$$\text{Gluconic acid}=CH_2(OH).[CH(OH)]_4.COOH;$$
$$\text{Saccharic acid}=COOH.[CH(OH)]_4.COOH.$$

The monobasic oxyacids are of the greatest importance in the artificial formation of the monosaccharides. These acids, as lactones, can be converted into their respective aldehydes (corresponding to the sugars) by the action of nascent hydrogen. On the other hand, they may be transformed into stereoisomeric acids on heating with quinoline, pyridine, etc., and the stereoisomeric sugars may be obtained from these by reduction.

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[1] Ber. d. deutsch. chem. Gesellsch. 18, 19, and 20.

[2] See E. Fischer's lecture, Synthesen in der Zuckergruppe, Ber. d. deutsch. chem. Gesellsch., 23, 2114. Excellent works on carbohydrates are Tollens' Kurzes Handbuch der Kohlehydrate, Breslau, 2, 1895, and 1, 2. Auflage, 1898, which gives a complete review of the literature, and E. O. v. Lippmann, Die Chemie der Zuckerarten, Braunschweig, 1904.

Numerous isomers occur among the monosaccharides, and especially in the hexose group. In certain cases, as for instance in glucose and levulose, we are dealing with a different constitution (aldoses and ketoses), but in most cases we have stereoisomerism due to the presence of asymmetric carbon atoms.

The monosaccharides are converted into the corresponding polyhydric alcohols by nascent hydrogen. Thus ARABINOSE, which is a pentose, $C_5H_{10}O_5$, is transformed into the pentatomic alcohol, ARABITE, $C_5H_{12}O_5$. The three hexoses, DEXTROSE, LEVULOSE, and GALACTOSE, $C_6H_{12}O_6$, are transformed into the corresponding three hexites, SORBITE, MANNITE, and DULCITE, $C_6H_{14}O_6$. In these reductions a second isomeric alcohol is also obtained; in the reduction of levulose we obtain besides mannite also sorbite. Inversely, the corresponding sugars may be prepared from polyhydric alcohols by careful oxidation.

Like the ordinary aldehydes and ketones, the sugars may be made to take up hydrocyanic acid. Cyanhydrins are thus formed. These addition products are of special interest in that they make possible the artificial preparation of sugars rich in carbon from sugars poor in carbon.

As an example, if we start from dextrose we obtain glucocyanhydrin on the addition of hydrocyanic acid:

$$CH_2.(OH).[CH(OH)]_4.COH + HCN = CH_2(OH).[CH(OH)]_4.CH(OH).CN.$$

On the saponification of glucocyanhydrin the corresponding oxyacid is formed:

$$\begin{aligned}&CH_2(OH).[CH(OH)]_4.CH(OH).CN + 2H_2O\\&\qquad = CH_2(OH).[CH(OH)]_4.CH(OH).COOH + NH_3.\end{aligned}$$

By the action of nascent hydrogen on the lactone of this acid we obtain glucoheptose, $C_7H_{14}O_7$.

The monosaccharides give the corresponding oximes with hydroxylamine; thus glucose yields glucosoxime, $CH_2(OH).[CH(OH)]_4.CH:N.OH$. These compounds are of importance on account of the fact, as found by WOHL,[1] that they are the starting-point in the formation of one class of sugars from another class, namely, the preparation of sugars poor in carbon from those rich in carbon.

The monosaccharides are strong reducing bodies, similar to the aldehydes. They reduce metallic silver from ammoniacal silver solutions, and also several metallic oxides, such as copper, bismuth, and mercury oxides, on warming their alkaline solutions. This property is of the greatest importance in their detection and quantitative estimation.

With phenylhydrazine or substituted phenylhydrazines, the sugars first yield *hydrazones* with the elimination of water, and then on the further action of hydrazine on warming in an acetic-acid solution we obtain *osazones*.

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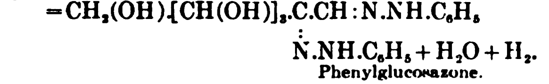
[1] Ber. d. d. chem. Gesellsch., **26**. p. 730.

The reaction takes place as follows:

(*a*) $CH_2(OH).[CH(OH)]_3.CH(OH).CHO + H_2N.NH.C_6H_5$
$= CH_2(OH).[CH(OH)]_3.CH(OH)CH:N.NH.C_6H_5 + H_2O.$
Phenylglucosehydrazone.



$$\begin{aligned}(b)\ & CH_2(OH)[CH(OH)]_3.CH(OH).CH:N.NH.C_6H_5 + H_2N.NH.C_6H_5 \\ & = CH_2(OH).[CH(OH)]_3.\underset{\vdots\atop N.NH.C_6H_5}{C}.CH:N.NH.C_6H_5 + H_2O + H_2.\end{aligned}$$

Phenylglucosazone.

The hydrogen is not evolved, but acts on a second molecule of phenylhydrazine and splits it into aniline and ammonia:

$$H_2N.NH.C_6H_5 + H_2 = H_2N.C_6H_5 + NH_3.$$

The osazones are generally yellow crystalline compounds which differ from each other in melting-point, solubility, and optical properties, and hence have been of great importance in the characterization of certain sugars. They have also become of extraordinarily great interest in the study of the carbohydrates for other reasons. Thus they are a very good means of precipitating sugars from solution in which they occur mixed with other bodies, and they are of the greatest importance in the artificial preparation of sugars. On cleavage, by the brief action of gentle heat and fuming hydrochloric acid (for disaccharides still better with benzaldehyde)[1] the osazones yield so-called *osones*, which on reduction yield aldoses or more often ketoses.

We can also pass from the osazones to the corresponding sugars (ketoses) in other ways, namely, by direct reduction of the osazones with acetic acid and zinc dust. The corresponding osamine is first formed (from phenylglucosazone we obtain isoglucosamine), which on treatment with nitrous acid yields the sugar (in this case levulose).

The sugars can be prepared from the hydrazones by decomposition with benzaldehyde (HERZFELD) or with formaldehyde (RUFF and OLLENDORFF[2]). This latter method is especially applicable if substituted hydrazines, especially benzylphenylhydrazine, are used.

With ammonia the glucoses may form compounds which have been considered as osamines by LOBRY DE BRUYN, but to differentiate them from the true osamines have been called osimines by E. FISCHER.[3] The corresponding osaminic acid can be obtained from such an osimine by the action of ammonia and hydrocyanic acid, and from the hydrochloric-acid lactone of this acid the osamine is obtained by reduction with sodium amalgam. In this manner E. FISCHER and LEUCHS artificially prepared *d*-glucosamine, which occurs in the animal kingdom and is an isomer of the above-mentioned isoglucosamine, by starting from *d*-arabinose, then obtaining *d*-arabinosimine, then *d*-glucosaminic acid, and finally the glucosamine from the lactone of this acid. They[1] also have prepared *l*-glucosamine from *l*-arabinose in a similar manner.

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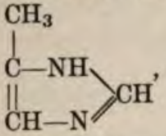
[1] E. Fischer and Armstrong, Ber. d. d. chem. Gesellsch., **35**.

[2] Herzfeld, *ibid.*, 28; Ruff and Ollendorff, *ibid.*, 32.

[3] Lobry de Bruyn, *ibid.*, 28; E. Fischer, *ibid.*, 35.

Knoop and Windaus[2] have obtained large amounts of methylimidazol,  , from glucose by the action of ammonium-zinc hydroxide at ordinary temperatures. A genetic relationship of the carbohydrates to histidine and the purine bodies is thus made probable by the imidazol formation.

By the action of hydrochloric acid upon alcoholic sugar solutions E. Fischer and his pupils have obtained ether-like compounds which have been called *glucosides*. Compounds with aromatic groups similar to the glucosides occur widely distributed in the vegetable kingdom. The more complex carbohydrates may be considered, according to Fischer, as glucosides of the sugars. Thus maltose, for example, is the glucoside and lactose the galactoside of dextrose.

By the action of alkalies, even in small amounts, as also of alkaline earths and lead hydroxide, a reciprocal transformation of the sugars, such as dextrose, levulose, and mannose, may take place (Lobry de Bruyn and Alberda van Ekenstein[3]).

Four other sugars, among them two ketoses, are produced by the action of potash or soda on each of the three sugars, dextrose, levulose, and galactose. For example, from dextrose two ketoses, levulose and pseudolevulose, are produced, also mannose and a non-fermentable sugar, glutose. From galactose are formed talose and galtose, besides two ketoses, tagatose and pseudotagatose.

The transformation of the different varieties of sugar into each other also occurs in the animal body. Neuberg and Mayer[4] have shown by experiments on rabbits the partial transformation of various mannoses into the corresponding glucoses.

The monosaccharides are colorless and odorless bodies, neutral in reaction, with a sweet taste, readily soluble in water, generally soluble with difficulty in absolute alcohol, and insoluble in ether. Some of them crystallize well in the pure state. They are optically active, some levorotatory and others dextrorotatory; but there are also optically inactive modifications (racemic), which are formed from two optically opposed components.

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[1] Ber. d. d. chem. Gesellsch., **35**, p. 3787, and **36**, p. 24.

[2] *Ibid.*, **38**, and Hofmeister's Beiträge, **6**.

[3] Ber. d. d. chem. Gesellsch., **28**, 3078; Bull. soc. chim. de Paris (3), **15**; Chem. Centralbl., 1896, **2**, and 1897, **2**.

[4] Zeitschr. f. physiol. Chem., **37**.

We designate the optical activity of the carbohydrates with the letter *l*- for levogyrate, *d*- for dextrogyrate, and *i*- for inactive. These are only partly indicative. Thus dextrorotatory glucose is designated *d*-glucose, levorotatory *l*-glucose, and the inactive *i*-glucose. EMIL FISCHER has used these signs in another sense. He designates by these signs the mutual relationship of the various kinds of sugars instead of their optical activity. For example, he does not designate the levorotatory levulose *l*-levulose, but *d*-levulose, showing its close relation to dextrorotatory *d*-glucose. This designation is generally accepted, and the above-mentioned signs only show the optical properties in certain cases.

Specific rotation means the rotation in degrees produced by 1 gm. substance dissolved in 1 c.c. liquid placed in a tube 1 dcm. long. The reading is ordinarily made at 20° C. and with the monochromatic sodium light. The specific rotation with this light is represented by $(\alpha)_D$, and is expressed by the following formula: $(\alpha)_D = \pm\frac{a}{p.1}$, in which a represents the reading of degrees, 1 the length of the tube in decimetres, and p the weight of substance in 1 c.c. of the liquid. Inversely the per cent P of substance can be calculated, when the specific rotation is known, by the formula $P=\frac{100a}{s.1}$, in which s represents the known specific rotation.

A freshly prepared sugar solution often shows a different rotation from one which has been allowed to stand for some time. If the rotation gradually diminishes, this is called birotation, while a gradual increase in the rotation is called half-rotation.

Many monosaccharides, but not all, ferment with yeast, and it has been shown that only those varieties of sugar containing 3, 6, or 9 atoms of carbon in the molecule are fermentable with yeast. We must state, however, that the power of fermentation with pure yeast has been shown only for the hexose group, and in fact all of the hexoses do not ferment. The restriction of fermentation to only certain monosaccharides is, according to E. FISCHER, like the action of the inverting enzymes upon disaccharides and glucosides, dependent upon the stereometric configuration of the sugars (see Chapter I). This difference in configuration is important not only for the action of lower living organisms upon the sugars, but also upon the behavior of the sugars within more highly developed organisms. Thus the investigations of NEUBERG and WOHLGEMUTH[1] upon arabinose and of NEUBERG and MAYER[2] on mannoses have shown that in rabbits the *l*-arabinose and the *d*-mannose are much better utilized than *d*- and *i*-arabinose or *l*- and *i*-mannose, and they have also shown that the lower organisms have the tendency toward decomposing inactive substances into their optically active components to a much higher degree than the higher organisms.

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[1] Zeitschr. f. physiol. Chem., 35.

[2] *Ibid.*, 37.

By the action of lower organisms of various kinds the sugars may be made to undergo fermentations of different kinds, such as lactic-acid and butyric-acid fermentation and mucilaginous fermentation.

The simple varieties of sugar occur in part in nature as such, already formed, which is the case with both of the very important sugars, dextrose and levulose. They also occur in great abundance in nature as more complex carbohydrates (di- and polysaccharides); also as ester-like combinations with different substances, as so-called glucosides.

Among the groups of monosaccharides known at the present time, those containing less than five and more than six carbon atoms in the molecule have no great importance in biochemistry, although they are of high scientific interest. Of the other two groups the hexoses are of the greatest importance, hence in the past only those carbohydrates with six carbon atoms were considered as true carbohydrates. As the pentoses have been the subject of numerous biochemical investigations of late, they will also be discussed in brief.

Pentoses ($C_5H_{10}O_5$).

As a rule the pentoses do not occur as such in nature, but are formed in the hydrolytic splitting of several more complex carbohydrates, the so-called *pentosanes*, especially on boiling gums with dilute mineral acids. The pentosanes exist very widely distributed in the plant kingdom and are especially of great importance in the building up of certain plant constituents. The pentoses were first found by SALKOWSKI and JASTROWITZ in the animal kingdom in the urine of a person addicted to the morphine habit, and later by SALKOWSKI and others in normal human urine. Small quantities of pentoses have been detected by KÜLZ and VOGEL[1] in the urine of diabetics, as also in dogs with pancreas diabetes or phlorhizin diabetes. Pentose has also been found by HAMMARSTEN amongst the cleavage products of a nucleoproteid obtained from the pancreas, and seems also, according to the observations of BLUMENTHAL, to be a constituent of nucleoproteids of various organs, such as the thymus, thyroid, brain, spleen, and liver. In regard to the quantity of pentoses found in the various organs, we must refer to the works of GRUND and of BENDIX and EBSTEIN.[2]

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[1] Salkowski and Jastrowitz, Centralbl. f. d. med. Wissensch., 1892, 337 and 593; Salkowski, Berl. klin. Wochenschr., 1895; Bial Zeitschr. f. klin. Med., **39**; Bial and Blumenthal, Deutsch. med. Wochenschr., 1901, No. 2; Külz and Vogel, Zeitschr. f. Biologie **32**.

[2] Hammarsten Zeitschr f. physiol. Chem., **19**; also Salkowski, Berl. klin. Wochenschr., 1895; Blumenthal Zeitschr. f. klin. Med., **34**; Grund, Zeitschr. f. physiol. Chem., **35**; Bendix and Ebstein, Zeitschr. f. allgemein. Physiol., **2**.

The pentosanes (Stone, Slowtzoff) as well as the pentoses are of the greatest importance as foods for herbivorous animals. In regard to the value of the pentoses, the researches of Salkowski, Cremer, Neuberg, and Wohlgemuth[1] upon rabbits and hens show that these animals can utilize the pentoses. The question whether the pentoses are active as glycogen-formers is still an open one (see Chapter VIII). The pentoses seem to be absorbed by human beings and in part utilized, but they pass in part into the urine even when small quantities are taken.[2]

The natural pentoses are reducing aldoses, and as a rule do not belong to the sugars fermentable with yeast. Still, the observations of Salkowski, Bendix, Schöne and Tollens seem to indicate that the pentoses are fermentable.[3] They are readily decomposed by putrefaction bacteria. With phenylhydrazine and acetic acid they give crystalline osazones which are soluble in hot water and whose melting-points and optical behavior are important for the detection of the pentoses. On heating with hydrochloric acid they yield furfurol, but no levulinic acid. The furfurol passing over on distilling with hydrochloric acid can be detected by the aid of aniline-acetate paper, which is colored beautifully red by furfurol. In the quantitative estimation we can use the method suggested by Tollens, which consists of converting the furfurol in the distillate into phloroglucide by means of phloroglucin and weighing (see Tollens and Kröber, Grund, Bendix and Ebstein).[4] These methods are still not quite accurate, to say nothing of the fact that glucuronic-acid compounds also yield furfurol under the same conditions. The two following pentose reactions, as suggested by Tollens, are especially applicable.

The orcin-hydrochloric acid test. Mix with the solution or the substance introduced into water an equal volume of concentrated hydrochloric acid, add some orcin in substance, and heat. In the presence of pentoses the solution becomes reddish blue, then bluish green, and on spectroscopic examination an absorption-band is observed between *C* and *D*. If it is cooled and shaken with amyl alcohol, a bluish-green solution which shows the same band is obtained.

The phloroglucin-hydrochloric acid test. This test is performed in the same manner as the above, using phloroglucin instead of orcin. The solution becomes cherry-red on heating and then becomes cloudy and hence a shaking out with amyl alcohol is very necessary. The red amyl-alcohol solution shows an absorption-band between *D* and *E*. The orcin test is better for several reasons than the phloroglucin test (Salkowski and Neuberg[1]). In regard to the use of these tests in urine examination see Chapter XV.

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[1] Stone, Amer. Chem. Journ., **14**; Slowtzoff, Zeitschr. f. physiol. Chem., **34**; Salkowski, l. c., Centralbl.; Cremer, Zeitschr. f. Biologie, **29** and **42**; Neuberg and Wohlgemuth, Zeitschr. f. physiol. Chem., **35**.

[2] See Ebstein, Virchow's Arch., **129**; Tollens, Ber. d. deutsch. chem. Gesellsch., **29**, 1208; Cremer, l. c.; Lindemann and May, Deutsch. Arch. f. klin. Med., **56**; Salkowski, Zeitschr. f. physiol. Chem.. **30**.

[3] Salkowski. Zeitschr. f. physiol. Chem., **30**; Bendix, see Chem. Centralbl., 1900, **1**; Schöne and Tollens. *ibid.*, 1901, **1**.

[4] Bendix and Ebstein, l. c., which contains the literature.

Many modifications of these tests have been suggested. Brat[2] performs the orcin reaction by the addition of NaCl and heating to only 90–95°. Bial[3] uses a hydrochloric acid containing ferric chloride for the orcin test and claims to get a greater delicacy. On too strong and too long heating (1½–2 minutes), when using this modification, a confusion with sugars of the six carbon series may occur (Bial, van Leersum).[4] According to R. Adler and O. Adler the phloroglucin and orcin tests can be made with glacial acetic acid and a few drops hydrochloric acid instead of with the hydrochloric acid alone. These investigators also use a mixture of equal volumes of aniline and glacial acetic acid as a reagent for pentoses. On the addition of a little pentose to the boiling mixture a beautiful red color of furfurol-aniline acetate is obtained. A. Neumann[5] performs the orcin test with glacial acetic acid and adds concentrated sulphuric acid drop by drop. On following the exact instructions not only do the pentoses give this reaction, but also glucuronic acid, dextrose, and levulose give characteristic colored solutions with special absorption-bands which can be made use of in identifying the various sugars.

In performing the above two tests for pentose it must be borne in mind that glucuronic acid gives the same reactions and also that the colors alone are not sufficient. The spectroscopic examination must therefore never be omitted. Both tests are to be considered as tests of detection rather than definite pentose reactions, and therefore for a positive detection of pentoses we must prepare also the osazones or other compounds.

Arabinoses. The pentose isolated by Neuberg[6] from human urine is *i*-arabinose. It can be isolated from the urine as the diphenylhydrazone, from which the arabinose can be separated by splitting with formaldehyde. The *i*-arabinose is crystalline, has a sweetish taste, is optically inactive, and melts at 163–164° C. Its diphenylhydrazone, which, according to Neuberg and Wohlgemuth,[7] can be used in its quantitative estimation, melts at 206° C., is insoluble in cold water and alcohol, but readily soluble in pyridine. The osazone melts at 166–168° C.

The dextrorotatory *l*-arabinose is obtained by boiling gum arabic or cherry gum with dilute sulphuric acid. The *d*-arabinose is prepared synthetically. The diphenylhydrazone of *l*-arabinose has according to Neuberg a melting-point of 216–218° C., while according to TOLLENS and MAURENBRECHER[1] its melting-point is 204–205°.

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[1] Salkowski, Zeitschr. f. physiol. Chem., **27**; Neuberg, *ibid.*, **31**.

[2] Zeitschr. f. klin. Med., **47**.

[3] Deutsch. med. Wochenschr., 1902 and 1903, and Zeitschr. f. klin. Med., **50**.

[4] Bial, Zeitschr. f. klin. Med., **50**; van Leersum, Hofmeister's Beiträge, **5**.

[5] R. and O. Adler, Pflüger's Arch., **106**; A. Neumann, Berl. klin. Wochenschr., 1904.

[6] Ber. d. d. chem. Gesellsch., **33**.

[7] Zeitschr. f. physiol. Chem., **35**.

Xyloses. The only pentose thus far isolated from the animal tissues is *l*-xylose, obtained by NEUBERG from the pancreas proteins, and is identical with the xylose found widely distributed in the plant kingdom and obtained from wood-gum by boiling with dilute acids. Xylose is crystalline, melts at 153–154° C., dissolves very readily in water but with difficulty in alcohol, is faintly dextrorotatory, $(a)_D = +18.1°$, and gives a phenylosazone which melts at 159–160° C., and according to TOLLENS and MÜTHER[2] a diphenylhydrazone which melts at 107–108°. Xylose can be transformed into xylonic acid, $CH_2OH(CHOH)_3COOH$, by bromine-water, and the brucine salt of this acid is, according to NEUBERG, well suited for the detection and isolation of xylose.

Hexoses ($C_6H_{12}O_6$).

The most important and best-known simple sugars belong to this group, and most of the other bodies which have been considered as carbohydrates in the past are anhydrides of this group. Certain hexoses, such as dextrose and levulose, either occur in nature already formed or are produced by the hydrolytic splitting of other more complicated carbohydrates or glucosides. Others, such as mannose or galactose, are formed by the hydrolytic cleavage of other natural products; while some, on the contrary, such as gulose, talose, and others, are obtained only by artificial means.

All hexoses, as also their anhydrides, yield levulinic acid, $C_5H_8O_3$, besides formic acid and humus substances on boiling with dilute mineral acids. Some of the hexoses are fermentable with yeast, while the artificially prepared hexoses are not, or at least only incompletely and with great difficulty.

Some hexoses are aldoses, while others are ketoses. Belonging to the first group we have MANNOSE, DEXTROSE, GULOSE, GALACTOSE, and TALOSE, and to the other LEVULOSE, and possibly also SORBINOSE. We differentiate also between the *d*, *l*, and *i* modifications; for instance, *d*-, *l*-, and *i*-dextrose; hence the number of isomers is very great.

The most important syntheses of the carbohydrates have been made by E. FISCHER and his pupils chiefly within the members of the hexose group. A short summary of the syntheses of hexoses is given below.

The first artificial preparation of a sugar was made by BUTLEROW. On treating trioxymethylene, a polymer of formaldehyde, with lime-water he obtained a faintly sweetish syrup called *methylenitan*. Loew[1] later obtained a mixture of several sugars, from which he isolated a fermentable sugar, called *methose*, by condensation of formaldehyde in the presence of bases. The most important and comprehensive syntheses of sugar have been performed by E. Fischer.[2]

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[1] Neuberg, Zeitschr. f. physiol. Chem., 35, and Ber. d. d. chem. Gesellsch., 33; Tollens and Maurenbrecher, *ibid.*, 38.

[2] Neuberg, Ber. d. d. chem. Gesellsch., 35; Tollens and Müther, *ibid.*, 37.

The starting-point of these syntheses is *α-acrose*, which occurs as a condensation product of formaldehyde. The name α-acrose has been given to this body because it is obtained from acrolein bromide by the action of bases (Fischer). It is also obtained admixed with *β-acrose* on the oxidation of glycerine with bromine in the presence of sodium carbonate and treating the resulting mixture with alkali. On the oxidation with bromine a mixture of glycerine aldehyde, $CH_2OH.CH(OH).CHO$, and dioxyacetone, $CH_2(OH).CO.CH_2OH$, is obtained. These two bodies may be considered as true sugars, namely, glyceroses or trioses. It seems as if a condensation to hexoses takes place on treatment with alkalies.

α-acrose may be isolated from the above mixture and obtained pure by first converting it into its osazone and then retransforming this into the sugar. α-acrose is identical with *i*-levulose. With yeast one half, the levogyrate *d*-levulose, ferments, while the dextrogyrate *l*-levulose remains. The *i*- and *l*-levulose may be prepared in this way.

On the reduction of α-acrose we obtain α-acrite, which is identical with *i*-mannite. On oxidation of *i*-mannite we obtain *i*-mannose, from which only *l*-mannose remains on fermentation. On further oxidation of *i*-mannose it yields *i*-mannonic acid. The two active mannonic acids may be separated from each other by the fractional crystallization of their strychnine or morphine salts. The two corresponding mannoses may be obtained from these two acids, *d*- and *l*-mannonic acids, by reduction.

d-Levulose is obtained from *d*-mannose by the method given on page 107, using the osazone as an intermediate step. The *d*- and *l*-mannonic acids are partly converted into *d*- and *l*-gluconic acids on heating with quinoline, and *d*- or *l*-glucose is obtained on the reduction of these acids; *l*-glucose is best prepared from *l*-arabinose by means of the cyanhydrin reaction, using *l*-gluconic acid as the intermediate step. The combination of *l*- and *d*-gluconic acids, forming *i*-gluconic acid, yields *i*-glucose on reduction.

The artificial preparation of sugars by means of the condensation of formaldehyde has received special interest because, according to Baeyer's assimilation hypothesis, in plants formaldehyde is first formed by the reduction of carbon dioxide, and the sugars are produced by the condensation of this formaldehyde. Bokorny[3] has shown, by special experiments on algæ Spirogyra, that formaldehyde sodium sulphite was split by the living algæ cells. The formaldehyde set free is immediately condensed to carbohydrate and precipitated as starch.

Among the hexoses known at the present time only dextrose, levulose and galactose are really of physiological-chemical interest; therefore the other hexoses will be only incidentally mentioned.

Dextrose (*d*-glucose)—GLUCOSE, GRAPE-SUGAR, and DIABETIC SUGAR—occurs abundantly in the grape, and also, often accompanied with levulose (*d*-fructose), in honey, sweet fruits, seeds, roots, etc. It occurs in the human and animal intestinal tract during digestion, also in small quantities in the blood and lymph, and as traces in other animal fluids and tissues.

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[1] Butlerow, Ann. d. Chem. u. Pharm., **120**; Compt. rend., **53**; O. Loew, Journ. f. prakt. Chem. (N. F.), **33**, and Ber. d. deutsch. chem. Gesellsch. **20**, **21**, **22**.

[2] Ber. d. d. chem. Gesellsch., **21**, and l. c., p. 105.

[3] Biolog. Centralbl., **12**, pp. 321 and 481.

It occurs only as traces in urine under normal conditions, while in diabetes the quantity is very large. It is formed in the hydrolytic cleavage of starch, dextrin, and other compound carbohydrates, as also in the splitting of glucosides. The question whether dextrose can be formed in the body from proteins or from fats is disputed and will be discussed in a following chapter (VIII).

Properties of Dextrose. Dextrose crystallizes sometimes with 1 molecule of water of crystallization in warty masses or small leaves or plates, and sometimes when free from water in needles or prisms. The sugar containing water of crystallization melts even below 100° C. and loses its water of crystallization at 110° C. The anhydrous sugar melts at 146° C., and is converted into glucosan, $C_6H_{10}O_5$, at 170° C. with the elimination of water. On strongly heating it is converted into caramel and then decomposes.

Dextrose is readily soluble in water. This solution, which is not as sweet as a cane-sugar solution of the same strength, is dextrogyrate and shows strong birotation. The specific rotation is dependent upon the concentration of the solution, as it increases with an increase in the concentration. A 10 per cent solution of anhydrous glucose can be taken as 52.74° at 20° C.[1] Dextrose dissolves sparingly in cold, but more freely in boiling alcohol. 100 parts alcohol of sp. gr. 0.837 dissolves 1.95 parts anhydrous dextrose at 17.5° C. and 27.7 parts at the boiling temperature (ANTHON[2]). Dextrose is insoluble in ether.

If an alcoholic caustic-potash solution is added to an alcoholic solution of dextrose, an amorphous precipitate of insoluble sugar-potash compound is formed. On warming this compound it decomposes easily with the formation of a yellow or brownish color, which is the basis of MOORE's test. Dextrose forms also compounds with lime and baryta.

MOORE's *Test.* If a dextrose solution is treated with about one quarter of its volume of caustic potash or soda and warmed, the solution becomes first yellow, then orange, yellowish brown, and lastly dark brown. It has at the same time a faint odor of caramel, and this odor is more pronounced on acidification.[3]

Dextrose forms several crystallizable combinations with NaCl, of which the easiest to obtain is $(C_6H_{12}O_6)_2.NaCl+H_2O$, which forms large colorless six-sided double pyramids or rhomboids with 13.52 per cent NaCl.

Dextrose in neutral or very faintly acid (organic acid) solution undergoes alcoholic fermentation with beer-yeast: $C_6H_{12}O_6=2C_2H_5.OH+2CO_2$. Besides the alcohol and carbon dioxide there are formed, especially at higher temperatures, small quantities of homologous alcohols (amyl alcohol), glycerine, and succinic acid. In the presence of acid milk or cheese the dextrose undergoes lactic-acid fermentation, especially in the presence of a base such as ZnO or $CaCO_3$. The lactic acid may then further undergo butyric-acid fermentation: $2C_3H_6O_3 = C_4H_8O_2 + 2CO_2 + 4H$.

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[1] For further information see Tollens, Handbuch der Kohlehydrate, 2. Aufl., 44.

[2] Cited from Tollens' Handbuch.

[3] In regard to the products formed in this reaction, see Framm, Pflüger's Arch., 64. and especially Gaud, Compt. rend., 119.

Dextrose reduces several metallic oxides, such as copper oxide, bismuth oxide and mercuric oxide, in alkaline solutions, and the most important reactions for sugar are based on this fact.

TROMMER's *test* is based on the property that dextrose possesses of reducing cupric hydrate in alkaline solution into cuprous oxide. Treat the dextrose solution with about $\frac{1}{5}$–$\frac{1}{8}$ vol. caustic soda and then carefully add a dilute copper-sulphate solution. The cupric hydrate is thereby dissolved, forming a beautiful blue solution, and the addition of copper sulphate is continued until a very small amount of hydrate remains undissolved in the liquid. This is now warmed, and a yellow hydrated suboxide or red suboxide separates even below the boiling temperature. If too little copper salt has been added, the test will be yellowish brown in color, as in MOORE's test; but if an excess of copper salt has been added, the excess of hydrate is converted on boiling into a dark-brown hydrate which interferes with the test. To prevent these difficulties the so-called FEHLING's solution may be employed. This solution is obtained by mixing just before use equal volumes of an alkaline solution of Rochelle salt and a copper-sulphate solution (see Quantitative Estimation of Sugar in the Urine in regard to concentration). This solution is not reduced or noticeably changed by boiling. The tartrate holds the excess of cupric hydrate in solution, and an excess of the reagent does not interfere in the performance of the test. In the presence of sugar this solution is reduced.

BÖTTGER-ALMÉN's *test* is based on the property dextrose possesses of reducing bismuth oxide in alkaline solution. The reagent best adapted for this purpose is obtained, according to NYLANDER's [1] modification of ALMÉN's original test, by dissolving 4 grams of Rochelle salt in 100 parts of 10 per cent caustic-soda solution and adding 2 grams of bismuth subnitrate and digesting on the water-bath until as much of the bismuth salt is dissolved as possible. If a dextrose solution is treated with about $\frac{1}{10}$ vol., or with a larger quantity of the solution when large quantities of sugar are present, and boiled for a few minutes, the solution becomes first yellow, then yellowish brown, and finally nearly black, and after a time a black deposit of bismuth (?) settles.

The property of dextrose of reducing an alkaline solution of mercury on boiling is the basis of KNAPP's reaction with alkaline mercuric cyanide and of SACHSSE's reaction with an alkaline potassium-mercuric iodide solution.

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[1] Zeitschr. f. physiol. Chem., 8.

On heating with PHENYLHYDRAZINE ACETATE a dextrose solution gives a precipitate consisting of fine yellow crystalline needles which are nearly insoluble in water but soluble in boiling alcohol, and which separate again on treating the alcoholic solution with water. The crystalline precipitate consists of *phenylglucosazone* (see page 107). This compound melts when pure at 204–205° C., dissolves readily in pyridine (0.25 gram in 1 gram), and precipitates again from this solution as crystals on the addition of benzene, ligroin, or ether. According to Neuberg[1] this behavior can be used in the purification of the osazone.

Dextrose is not precipitated by a lead-acetate solution, but is almost completely precipitated by a solution of ammoniacal basic lead acetate. On warming, the precipitate becomes flesh-color or rose-red (Rubner's *reaction*[2]).

If a watery solution of dextrose is treated with *benzoylchloride* and an excess of caustic soda, and shaken until the odor of benzoylchloride has disappeared, a precipitate of benzoic-acid ester of dextrose will be produced which is insoluble in water or alkali (Baumann[3]).

If ½–1 c.c. of a dilute watery solution of dextrose is treated with a few drops of a 10 per cent alcoholic solution (free from acetone) of *α-naphthol*, the liquid is colored a beautiful violet on the addition of 1–2 c.c. of concentrated sulphuric acid (Molisch). According to Reinbold[4] this reaction depends first upon the formation of a volatile substance which gives a bluish-violet color with α-naphthol and sulphuric acid in the warmth. On further heating furfurol is also produced, which gives a raspberry-red to ruby-red coloration.

DIAZOBENZENESULPHONIC ACID gives with a dextrose solution made alkaline with a fixed alkali a red color, which after 10–15 minutes gradually changes to violet. ORTHONITROPHENYLPROPIOLIC ACID yields indigo when boiled with a small quantity of dextrose and sodium carbonate, and this is converted into indigo-white by an excess of sugar. An alkaline solution of dextrose is colored deep red on being warmed with a dilute solution of PICRIC ACID. The behavior of dextrose towards certain pentose reactions has already been given on page 112.

A more complete description as to the performance of these several tests will be given in detail in a subsequent chapter (on the urine).

Dextrose is prepared pure by inverting cane-sugar by the following simple method of Soxhlet and Tollens, being a modification of Schwarz's[5] method:

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[1] Ber. d. d. chem. Gesellsch., **32**, 3384.

[2] Zeitschr. f. Biologie, **20**.

[3] Ber. d. deutsch. chem. Gesellsch., **19**; also Kueny, Zeitschr. f. physiol. Chem., **14**, and Skraup, Wien. Sitzungsber., **98** (1888).

[4] Molisch, Monatshefte f. Chem., **7**, and Centralbl. f. d. med. Wissensch., 1887, pp. 34 and 49; Reinbold, Pflüger's Arch., **103**.

[5] Tollens, Handbuch der Kohlehydrate, 2. Aufl. I, 39.

Treat 12 litres 90 per cent alcohol with 480 c.c. fuming hydrochloric acid and warm to 45–50° C.: gradually add 4 kilos of powdered cane-sugar, and allow to cool after two hours, when all the sugar will have dissolved and been inverted. To incite crystallization, some crystals of anhydrous dextrose are added, and after several days the crystals are sucked dry by the air-pump, washed with dilute alcohol to remove hydrochloric acid, and crystallized from alcohol or methyl alcohol. According to TOLLENS it is, best to dissolve the sugar in one half its weight of water on the water-bath and then add double this volume of 90–95 per cent alcohol.

In detecting dextrose in animal fluids or extracts of tissues we may make use of the above-mentioned reduction tests, the optical determination, fermentation, and phenylhydrazine tests. For the quantitative estimation the reader is referred to the chapter on the urine. Those liquids containing proteins must first have these removed by coagulation with heat and addition of acetic acid, or by precipitation with alcohol or metallic salts, before testing for dextrose. In regard to the difficulties of operating with blood and serous fluids we refer the student to the works of SCHENCK, RÖHMANN, ABELES, and SEEGEN.[1]

The **guloses** are stereoisomers of dextrose and may be prepared artificially. *d*-Gulose is obtained on the reduction of *d*-gulonic acid, which is obtained on the reduction of glucuronic acid.

Mannoses.—*d-Mannose*, also called *seminose*, is obtained with *d*-levulose on the careful oxidation of *d*-mannite. It is also obtained on the hydrolysis of natural carbohydrates, such as salep sli meand reserve cellulose (especially from the shavings from the ivory-nut). It is dextrorotatory, readily ferments with beer-yeast, gives a hydrazone not readily soluble in water, and an osazone which is identical with that from *d*-glucose.

Levulose, also called *d*-FRUCTOSE and FRUIT-SUGAR, occurs, as above stated, mixed with dextrose extensively distributed in the vegetable kingdom and also in honey. It is formed in the hydrolytic cleavage of cane-sugar and several other carbohydrates, but it is especially readily obtained by the hydrolytic splitting of inulin. In extraordinary cases of diabetes mellitus we find levulose in the urine. NEUBERG and STRAUSS[2] have detected levulose with positiveness in human blood-serum and exudates in certain cases.

Levulose crystallizes with difficulty in needles partly anhydrous and partly containing water. It is readily soluble in water, but nearly insoluble in cold absolute alcohol, though rather readily in boiling alcohol. Its aqueous solution is levogyrate. Levulose ferments with yeast, and gives the same reduction tests as dextrose, and also the same osazone. It gives a compound with lime which is less soluble than the corresponding dextrose compound. Levulose is not precipitated by sugar of lead or basic lead acetate.

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[1] Schenck, Pflüger's Arch., **46** and **47**; Röhmann, Centralbl. f. Physiol., **4**; Abeles, Zeitschr. f. physiol. Chem., **15**; Seegen, Centralbl. f. Physiol., **4**.

[2] Zeitschr. f. physiol. Chem., **36**, which also contains the older literature.

Levulose does not reduce copper to the same extent as dextrose. Under similar conditions the reduction relationship of dextrose to levulose is 100:92.08.

In detecting levulose and those varieties of sugar which yield levulose on cleavage we make use of the following reaction suggested by SELIWANOFF. To a few cubic centimetres of fuming hydrochloric acid, add an equal volume of water and a small quantity of the sugar solution or of the solid substance and a few crystals of resorcin and apply heat. The liquid becomes a beautiful red, and gradually a substance precipitates which is red in color and soluble in alcohol. According to OFNER[1] the mixture must not contain more than 12 per cent HCl, and the boiling must not be continued longer than twenty seconds, otherwise glucose, mannose, and indeed maltose, may give a similar reaction. R. and O. ADLER[2] perform the test with glacial acetic acid and a drop of hydrochloric acid and some resorcin, in which case a reaction with aldoses is not obtained. SELIWANOFF'S reaction, which according to ROSIN may be made more delicate by a combination with the spectroscopic examination, is, as NEUBERG[3] has shown, a general reaction for ketoses.

According to NEUBERG,[4] methylphenylhydrazine is an excellent substance to use for the separation and detection of levulose, as it gives a characteristic levulose-methylphenylosazone. This osazone when recrystallized from alcohol melts at 153°. It shows a dextrorotation of 1° 40′ when 0.2 gram of the osazone is dissolved in 4 c.c. pyridine and 6 c.c. absolute alcohol.

OFNER has made objections to the use of methylphenylhydrazine in the detection of levulose. He has obtained the osazone from dextrose and methylphenylhydrazine, although the osazone is formed much more quickly with levulose than with dextrose. Only when the separation of the osazone crystals with methylphenylhydrazine after the addition of acetic acid takes place within five hours at ordinary temperatures is the presence of levulose positively proven (OFNER[5]).

The use of secondary asymmetric hydrazines as a general reagent for ketoses and as a means of separation from aldoses is objected to by OFNER.

Levulose, as above stated, is best obtained by the hydrolytic cleavage of inulin, by warming with faintly acidulated water.

Sorbinose (sorbin) is a ketose obtained from the juice of the berry of the mountain ash under certain conditions. It is crystalline and levogyrate, and is converted into sorbite by reduction.

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[1] Monatshefte f. Chem., **25**.

[2] See foot-note 5, p. 112.

[3] Zeitschr. f. physiol. Chem., **31**; Rosin, *ibid.*, **38**.

[4] Ber. d. d. chem. Gesellsch., **35**; also Neuberg and Strauss, *ibid.*, **36**.

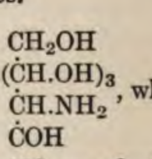
[5] *Ibid.*, **37**, and Zeitschr. f. physiol. Chem., **45**.

Galactose (not to be mistaken for lactose or milk-sugar) is obtained on the hydrolytic cleavage of milk-sugar and by hydrolysis of many other carbohydrates, especially varieties of gums and mucilaginous bodies. It is also obtained on heating cerebrin, a nitrogenized glucoside prepared from the brain, with dilute mineral acids.

It crystallizes in needles or leaves which melt at 168° C. It is somewhat less soluble than dextrose in water. It is dextrogyrate and shows multirotation. With ordinary yeast the galactose is slowly, but nevertheless completely, fermented. It is fermented by a great variety of yeasts (E. FISCHER and THIERFELDER), but not by Saccharomyces apiculatus,[1] which is of importance in physiological-chemical investigations. Galactose reduces FEHLING'S solution to a less extent than dextrose, and 10 c.c. of this solution are reduced, according to SOXHLET, by 0.0511 gram galactose in 1 per cent solution. Its phenylosazone melts at 193° C., and is soluble with difficulty in water, but with relative ease in hot alcohol. Its solution in glacial acetic acid is optically inactive. In the test with hydrochloric acid and phloroglucin galactose gives a color similar to the pentoses, but the solution does not give the absorption spectrum. On oxidation it first yields galactonic acid and then mucic acid. Both *l*- and *i*-galactose have been artificially prepared.

Talose is a sugar which is artificially prepared by the reduction of talonic acid. Talonic acid is obtained from *d*-galactonic acid by heating it with quinoline or pyridine to 140–150° C.

Appendix to the Hexoses.

α-Glucosamine[2] (chitosamine), $C_6H_{13}NO_5=$  $\begin{array}{c} CH_2OH \\ \dot{C}H.OH)_3 \\ \dot{C}H.NH_2 \\ \dot{C}OH \end{array}$, whose synthetical preparation has already been given on page 108, was first prepared by LEDDERHOSE[3] from chitin by the action of concentrated hydrochloric acid. Recently it has been obtained as a cleavage product of several mucin substances and proteins (see pages 33 and 65). Glucosamine is, as E. FISCHER and LEUCHS[4] have shown, a derivative of glucose or *d*-mannose (probably dextrose), and as an intermediary member between the hexoses and the oxyamino-acids obtainable from the proteins, it forms in certain regards a bridge between the proteins and the carbohydrates.

The free base is readily soluble in water with an alkaline reaction and quickly decomposes. The characteristic hydrochloride forms colorless crystals which are stable in the air and readily soluble in water, difficultly soluble in alcohol, and insoluble in ether. The solution is dextrorotatory, $(\alpha)_D = +70.15°$ to $74.64°$, at various concentrations.[1] Glucosamine has a reducing action similar to glucose, gives the same osazone, but is not fermentable. With benzoylchloride and caustic soda it gives a crystalline ester. In alkaline solution it gives with phenylisocyanate a compound which can be converted into its anhydride by acetic acid, and is used in the separation and detection of glucosamine (STEUDEL[2]). On oxidation with nitric acid it yields norisosaccharic acid, whose lead salt can be separated and whose salts with cinchonine or quinine are difficultly soluble in water and can also be used very successfully in the detection of glucosamine (NEUBERG and WOLFF[3]). On oxidation with bromine chitaminic acid (*d*-glucosaminic acid) is produced, and this is converted into chitaric acid, $C_6H_{10}O_6$, by nitrous acid. On treatment with nitrous acid glucosamine yields a non-fermentable sugar called chitose.

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[1] See F. Voit, Zeitschr. f. Biologie, **28** and **29**.

[2] According to E. Fischer's suggestion we shall use the term **glucosamine instead** of the term chitosamine which has lately been generally used.

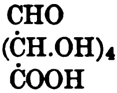
[3] Zeitschr. f. physiol. Chem., **2** and **4**.

[4] Ber. d. d. chem. Gesellsch., **36**.

EHRLICH[4] has suggested a test which does not respond with the free glucosamine, but with the mucins and other protein bodies containing an acetylated glucosamine. It consists in warming the substance, which has previously been treated with alkali, with a hydrochloric-acid solution of dimethylaminobenzaldehyde, when a beautiful red color is obtained.

Glucosamine is best prepared from decalcified lobster-shells by treating with hot concentrated hydrochloric acid.[5] In regard to its preparation from protein substances we must refer to the works cited on page 33, footnote 1.

Galactosamine has been prepared by SCHULZ and DITTHORN[6] from a glucoproteid of the spawn of the frog.



Glucuronic acid (glycuronic acid), $C_6H_{10}O_7 = \begin{matrix} CHO \\ (\dot{C}H.OH)_4 \\ \dot{C}OOH \end{matrix}$, is a derivative of dextrose and has been synthetically prepared by E. FISCHER and PILOTY[7] by the reduction of the lactone of saccharic acid. On oxidation with bromine it forms saccharic acid, and on reduction it yields gulonic-acid lactone. SALKOWSKI and NEUBERG[8] have obtained *l*-xylose from glucuronic acid by splitting off CO_2 by means of putrefaction bacteria.

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[1] See Hoppe-Seyler-Thierfelder's Handbuch, 7. Aufl.; Sundwik, Zeitschr. f. physiol. Chem., 34.

[2] Zeitschr. f. physiol. Chem., 34.

[3] Ber. d. d. chem. Gesellsch., 34.

[4] Mediz. Woche, 1901, No. 15; see Langstein, Ergebnisse der Physiol., I, Abt. 1, 88.

[5] See Hoppe-Seyler-Thierfelder's Handbuch, 7. Aufl.

[6] Zeitschr. f. physiol. Chem., 29.

[7] Ber. d. d. chem. Gesellsch., 24.

[8] Zeitschr. f. physiol. Chem., 36.

Glucuronic acid has not been found in the free state in the animal body. It occurs to a slight extent in normal urine as a conjugated acid, phenol- and probably also indoxyl- and skatoxylglucuronic acid (Mayer and Neuberg). It occurs to a much greater extent in urine as conjugated acid after the ingestion of certain aromatic and also aliphatic substances, especially camphor and chloral hydrate. It was obtained first by Schmiedeberg and Meyer from camphoglucuronic acid, and then by v. Mering[1] from urochloralic acid by cleavage with dilute acids. According to P. Mayer,[2] on the oxidation of dextrose a partial formation of glucuronic acid and oxalic acid takes place, and therefore, according to him, an increased elimination of conjugated glucuronic acids shows in certain cases an incomplete oxidation of dextrose. Conjugated glucuronic acids may also occur in the blood (P. Mayer; Lépine and Boulud[3]), in the fæces and in the bile.[4] Neuberg and Neimann[5] have prepared certain conjugated glucuronic acids (see Chapter XV) synthetically, among them being euxanthic acid. The most abundant source of glucuronic acid is the artist's pigment "Jaune indien," which contains the magnesium salt of euxanthic acid (euxanthon-glucuronic acid).

Glucuronic acid is not crystalline, but is only obtainable as a syrup. It dissolves in alcohol and is readily soluble in water. If the aqueous solution is boiled for an hour the acid is partly (20 per cent) converted into the crystalline lactone, glucurone, $C_6H_8O_6$, which is soluble in water and insoluble in alcohol. The alkali salts of the acid are crystalline. If a concentrated solution of the acid is saturated with barium hydrate the basic barium salt is obtained as a precipitate. The neutral lead salt is soluble in water, while the basic salt is insoluble. The readily crystallizable cinchonine salt can be used in isolating glucuronic acid (Neuberg[6]). Glucuronic acid is dextrorotatory, while the conjugated acids are levorotatory; they behave like dextrose with the reduction tests and do not ferment with yeast. They give the pentose reactions with phloroglucin or orcin and hydrochloric acid, and yield abundant furfurol on distillation with hydrochloric acid. With the phenylhydrazine test they give crystalline compounds which are not sufficiently characteristic (Thierfelder, P. Mayer[7]). By the action of 3 mol. phenylhydrazine and the necessary amount of acetic acid upon 1 mol. glucuronic acid at 40° for a few days

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[1] Mayer and Neuberg, Zeitschr. f. physiol. Chem., **29**; Schmiedeberg u. Meyer, *ibid.*, **3**; v. Mering, *ibid.*, 6.

[2] Zeitschr. f. klin. Med., **47**. See Chapter XV.

[3] Zeitschr. f. physiol. Chem., **32**; Lépine and Boulud, Compt. rend., **133, 134, 138**.

[4] See Bial, Hofmeister's Beiträge, **2**, and v. Leersum *ibid.*, **3**.

[5] Zeitschr. f. physiol. Chem., **44**.

[6] Ber. d. d. chem. Gesellsch., **33**.

[7] Thierfelder, Zeitschr. f. physiol. Chem., **11, 13, 15**; P. Mayer, *ibid.*, **29**.

Neuberg and Neimann obtained the glucuronic-acid osazone, which was very similar to glucosazone and melted at 200–205°. With *p*-bromphenylhydrazine hydrochloride and sodium acetate, glucuronic acid gives *p*-bromphenylhydrazine glucuronate, which is characterized by insolubility in absolute alcohol and by a very prominent levorotatory action. This compound is very well suited for the detection of glucuronic acid.[1] Dissolved in a mixture of alcohol and pyridine (0.2 gram substance in 4 c.c. pyridine and 6 c.c. alcohol) the rotation is 7° 25′, which corresponds to $(\alpha)_D^{20} = -369°$.

Glucuronic acid is best prepared from euxanthic acid, which decomposes by heating it with water to 120° C. for several hours. The filtrate from the euxanthon is concentrated at 40° C., when the anhydride gradually crystallizes out. On boiling the mother-liquor for some time and evaporating further, the crystals of the lactone are obtained. In regard to the quantitative estimation of glucuronic acid we must refer the reader to the works of Tollens and his collaborators and of Neuberg and Neimann.[2]

Disaccharides.

Some of the varieties of sugar belonging to this group occur ready formed in nature. Thus we have saccharose and lactose. Some, on the contrary, such as maltose and isomaltose, are produced by the partial hydrolytic cleavage of complicated carbohydrates. Isomaltose is besides this also obtained from dextrose by reversion (see page 125).

The disaccharides or hexobioses are to be considered as anhydrides, derived from two monosaccharides with the exit of 1 molecule of water. Corresponding to this, their general formula is $C_{12}H_{22}O_{11}$. On hydrolytic cleavage and the addition of water they yield 2 molecules of hexoses, either 2 molecules of the same hexose or one each of two different hexoses. Thus

$$\begin{aligned}&\text{Saccharose} + H_2O = \text{dextrose} + \text{levulose};\\&\text{Maltose} \quad + H_2O = \text{dextrose} + \text{dextrose};\\&\text{Lactose} \quad + H_2O = \text{dextrose} + \text{galactose}.\end{aligned}$$

The levulose turns the polarized ray more to the left than the dextrose does to the right; hence the mixture of hexoses obtained on the cleavage of saccharose has an opposite rotation to the saccharose itself. On this account the mixture is called INVERT-SUGAR, and the hydrolytic splitting is designated as *inversion*. This term inversion is not only used for the splitting of saccharose, but is also used for the hydrolytic cleavage of compound sugars into monosaccharides. The reverse reaction, whereby monosaccharides are condensed into complex carbohydrates, is called *reversion*.

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[1] See Neuberg, Ber. d. d. chem. Gesellsch., **32**, and Mayer and Neuberg, Zeitschr. f. physiol. Chem., **29**.

[2] Tollens, Zeitschr. f. physiol. Chem., **44**, which cites *also* the older work; Neuberg and Neimann, *ibid.*, **44**; Neuberg, *ibid.*, **45**.

We subdivide the disaccharides into two groups: first, the group to which saccharose belongs, where the members do not have the property of reducing certain metallic oxides; and the second group, to which the two maltoses and lactose belong, the members acting like monosaccharides in regard to the ordinary reduction tests. The members of the latter group have the character of aldehyde alcohols.

Saccharose, or CANE-SUGAR, occurs extensively distributed in the plant kingdom. It occurs to the greatest extent in the stalk of the sugar-millet and sugar-cane, the roots of the sugar-beet, the trunks of certain varieties of palms and maples, in carrots, etc. Cane-sugar is of extraordinarily great importance as a food and condiment.

Saccharose forms large, colorless monoclinic crystals. On heating it melts in the neighborhood of 160° C., and on heating more strongly it turns brown, forming so-called caramel. It dissolves very readily in water, and according to SCHEIBLER[1] 100 parts of saturated saccharose solution contain 67 parts of sugar at 20° C. It dissolves with difficulty in strong alcohol. Cane-sugar is strongly dextrorotatory. The specific rotation is only slightly modified by concentration, but is markedly changed by the presence of other inactive substances. The specific rotation is $(\alpha)_D = +66.5°$.

Saccharose acts indifferently towards MOORE's test and to the ordinary reduction tests. It does not ferment directly, but only after inversion, which can be brought about by an enzyme (invertin) contained in the yeast. An inversion of cane-sugar also takes place in the intestinal canal. Concentrated sulphuric acid blackens cane-sugar very quickly even at the ordinary temperature, and anhydrous oxalic acid does the same on warming on the water-bath. Various products are obtained on the oxidation of cane-sugar, dependent upon the variety of oxidizing material and also upon the intensity of the action. Saccharic acid and oxalic acid are the most important products.

The reader is referred to complete text-books on chemistry for the preparation and quantitative estimation of cane-sugar.

Maltose (MALT-SUGAR) is formed in the hydrolytic cleavage of starch by malt diastase, saliva, and pancreatic juice. It is obtained from glycogen under the same conditions (see Chapter VIII). Maltose is also produced transitorily in the action of sulphuric acid on starch. Maltose forms the fermentable sugar of the potato or grain mash, and also of the beerwort.

Maltose crystallizes with 1 molecule water of crystallization in fine white needles. It is readily soluble in water, rather easily in alcohol, but insoluble in ether. Its solutions are dextrorotatory; and the specific rotation is variable, depending upon the concentration and temperature, but is considerably stronger than dextrose.[1] Maltose ferments readily and completely with yeast, and acts like dextrose in regard to the reduction tests. It yields phenylmaltosazone on warming with phenylhydrazine for 1½ hours. This phenylmaltosazone melts at 206° C. and is more soluble than the glucosazone. Maltose differs from dextrose chiefly in the following: It does not dissolve as readily in alcohol, has a stronger dextrorotatory power, and has a feebler reducing action on FEHLING'S solution. 10 c.c. FEHLING'S solution is, according to SOXHLET,[2] reduced by 77.8 milligrams anhydrous maltose in approximately 1 per cent solution.

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[1] See Tollens' Handbuch der Kohlehydrate, 2. Aufl. 1, 124.

Isomaltose. This variety of sugar, as has been shown by FISCHER,[3] is produced, besides dextrin-like products, by reversion and by the action of fuming hydrochloric acid on dextrose. A re-formation of isomaltose and other sugars from dextrose can also be brought about by means of yeast maltase (HILL and EMMERLING[4]). It is also formed, besides ordinary maltose, in the action of diastase on starch paste, and occurs in beer and in commercial starch-sugar. The formation of isomaltose in the hydrolysis of starch by malt diastase has been denied by many investigators because they considered isomaltose as contaminated maltose.[5] It is also produced, with maltose, by the action of saliva or pancreatic juice (KÜLZ and VOGEL) or blood-serum (RÖHMANN[6]) on starch.

Isomaltose dissolves very readily in water, has a pronounced sweetish taste, and does not ferment, or, according to some, only very slowly. It is dextrorotatory, and has very nearly the same power of rotation as maltose. Isomaltose is characterized by its osazone. This forms fine yellow needles, which begin to form drops at 140° C. and melt at 150-153° C. It is rather easily soluble in hot water and dissolves in hot absolute alcohol much more readily than the maltosazone. Isomaltose reduces copper as well as bismuth solutions.

Lactose (MILK-SUGAR). As this sugar occurs exclusively in the animal world, in the milk of human beings and animals, it will be treated in a following chapter (on milk).

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[1] See Hoppe-Seyler-Thierfelder's Handbuch, 7. Aufl.

[2] Cited from Tollens' Handbuch der Kohlehydrate, 2. Aufl. 1, 154.

[3] Ber. d. deutsch. chem. Gesellsch., 23 and 28.

[4] Emmerling, *ibid.*, 34; Hill, *ibid.*, 34, and l. c., foot-note 1, p. 16.

[5] Brown and Morris, Journ. of Chem. Soc., 1895; Chem. News, 72. See also Ost, Ulrich, and Jalowetz, Ref. in Ber. d. deutsch. chem. Gesellsch., 28; Ling and Baker, Journ. of Chem. Soc., 1895; Pottevin, Chem. Centralbl., 1899, II, 1023.

[6] Külz and Vogel, Zeitschr. f. Biologie, 31; Röhmann, Centralbl. f. d. med. Wissensch., 1893, 849.

Polysaccharides.

If we exclude the hexotrioses and the few remaining sugar-like polysaccharides, this group includes a great number of very complex carbohydrates, which occur only in the amorphous condition, or at least not as crystals in the ordinary sense. Unlike the bodies belonging to the other groups, these have no sweet taste. Some are soluble in water, while others swell up therein, especially in warm water, and finally are neither dissolved nor visibly changed. Polysaccharides are ultimately converted into monosaccharides by hydrolytic cleavage.

The polysaccharides (not sugar-like) are ordinarily divided into the following chief groups: *starch group, gum* and *vegetable-mucilage group,* and *cellulose group.*

Starch Group, $(C_6H_{10}O_5)x$.

Starch, AMYLUM, $(C_6H_{10}O_5)x$. This substance occurs in the plant kingdom very extensively distributed in the different parts of the plant, especially as reserve food in the seeds, roots, tubers, and trunks.

Starch is a white, odorless, and tasteless powder, consisting of small granules which have a stratified structure and different shape and size in different plants. According to the ordinary opinion the starch granules consist of two different substances, STARCH GRANULOSE and STARCH CELLULOSE (v. NÄGELI), corresponding to MAQUENNE and ROUX'S[1] *amylose* and *amylopectin,* of which the first alone is converted into sugar on treatment with diastatic enzymes.

Starch is considered insoluble in cold water. The grains swell up in warm water and burst, yielding a paste. Starch is insoluble in alcohol and ether. On heating starch with water alone, or heating with glycerine to 190° C., or on treating the starch grains with 6 parts dilute hydrochloric acid of sp. gr. 1.07 at ordinary temperature for six to eight weeks,[2] it is converted into soluble starch (AMYLODEXTRIN, AMIDULIN). Soluble starch is also formed as an intermediate step in the conversion of starch into sugar by dilute acids or diastatic enzymes. Soluble starch may be precipitated from very dilute solutions by baryta-water.[3]

Starch granules swell up and form a pasty mass in caustic potash or soda. This mass gives neither MOORE'S nor TROMMER'S test. Starch paste does not ferment with yeast. The most characteristic test for starch is the blue coloration produced by iodine in the presence of hydriodic acid or alkali iodides.[1] This blue coloration disappears on the addition of alcohol or alkalies, and also on warming, but reappears again on cooling.

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[1] v. Nägeli, Botan. Mitteil., 1863; Maquenne and Roux, Compt. rend., **140**, and Bull. Soc. chim. de Paris **(3)**, **33.**

[2] See Tollens' Handb., 191. In regard to other methods, see Wróblewsky, Ber. d. deutsch. chem. Gesellsch., **30**; Syniewski, *ibid.*

[3] In regard to the compounds of soluble starch and dextrins with barium hydrate, see Bülow, Pflüger's Arch., **62.**

On boiling with dilute acids starch is converted into dextrose. In the conversion by means of diastatic enzymes we have as a rule, besides dextrin, maltose, and isomaltose, only very little dextrose. We are considerably in the dark as to the kind and number of intermediate products produced in this process (see Dextrins).

Starch may be detected by means of the microscope and by the iodine reaction. Starch is quantitatively estimated, according to SACHSSE'S method,[2] by converting it into dextrose by hydrochloric acid and then determining the dextrose by the ordinary methods.

Inulin, $(C_6H_{10}O_5)x+H_2O$, occurs in the underground parts of many compositæ, especially in the roots of the Inula helenium, the tubers of the dahlia, the varieties of helianthus, etc. It is ordinarily obtained from the tubers of the dahlia.

Inulin forms a white powder similar to starch, consisting of spheroid crystals which are readily soluble in warm water, without forming a paste. It separates slowly on cooling, but more rapidly on freezing. Its solutions are levogyrate and are precipitated by alcohol, and are colored only yellow with iodine. Inulin is converted into the levogyrate monosaccharide levulose on boiling with dilute sulphuric acid. Diastatic enzymes have no or only very slight action on inulin.[3]

According to DEAN[4] inulin occurs together with other substances, *levulins*, which are more soluble and have less rotation. He suggests that we limit the name inulin to that of carbohydrate (or mixture of carbohydrates), which is readily precipitable by 60 per cent alcohol and shows a specific rotation of $(\alpha)_D = -38\text{–}40°$.

Lichenin (MOSS-STARCH) occurs in many lichens, especially in Iceland moss. It is not soluble in cold water, but swells up into a jelly. It is soluble in hot water, forming a jelly on allowing the concentrated solution to cool. It is colored yellow by iodine and yields glucose on boiling with dilute acids. Lichenin is not changed by diastatic enzymes such as ptyalin or amylopsin (NILSON[5]).

Glycogen. This carbohydrate, which stands to a certain extent between starch and dextrin, is principally found in the animal kingdom, hence it will be considered in a subsequent chapter (on the liver).

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[1] See Mylius, Ber. d. deutsch. chem. Gesellsch., 20, and Zeitschr. f. physiol. Chem., 11.

[2] Tollens' Handb., 2. Aufl. 1, 187.

[3] *Ibid.*, 208.

[4] Amer. Chem. Journ., 32.

[5] Upsala Läkaref. Förh., 28.

The Gums and Vegetable Mucilages, $(C_6H_{10}O_5)x$.

These bodies may be divided into two chief groups, according to their origin and occurrence, namely, the *dextrin group* and the *vegetable gums* or *mucilages*. The dextrins stand in close relationship to the starches and are formed therefrom as intermediate products in the action of acids and diastatic enzymes. The various kinds of vegetable gums and vegetable mucilages occur, on the contrary, as natural products in the vegetable kingdom, and some may be separated from certain plants as amorphous, transparent masses, and others may be extracted from certain parts of the plant, such as the wood and seeds, by proper solvents.

The dextrins yield as final products only hexoses, indeed only dextrose, on complete hydrolysis. The vegetable gums and the mucilages yield, on the contrary, not only hexoses, but also an abundance of pentoses (gum arabic and wood-gum). *d*-Galactose occurs often among the hexoses, and accordingly as a differentiation from the dextrins, they yield mucic acid on oxidation with nitric acid. The dextrins, as well as the ordinary varieties of gums and mucilages, are precipitated by alcohol. Basic lead acetate precipitates the gums and mucilages, but not the dextrins.

Dextrin (starch-gum, British gum) is produced on heating starch to 200–210° C., or by heating starch, which has previously been moistened with water containing a little nitric acid, to 100–110° C. Dextrins are also produced by the action of dilute acids and diastatic enzymes on starch. We are not quite clear in regard to the steps taking place in the above processes, but the ordinary views are as follows: The first product, which gives a blue with iodine, is soluble starch or *amylodextrin*, which on further hydrolytic cleavage yields sugar and *erythrodextrin*, which is colored red by iodine. On further cleavage of this erythrodextrin more sugar and a dextrin, *achroodextrin*, which is not colored by iodine, are formed. From this achroodextrin after successive splittings we have sugar and dextrins of lower molecular weights formed, until finally we have sugar and a dextrin, *maltodextrin*, which refuses to split further, as final products. The views are rather contradictory in regard to the number of dextrins which occur as intermediate steps. The sugar formed is isomaltose, from which maltose and only very little dextrose are produced. Another view is that first several dextrins are formed consecutively in the successive splittings, with hydration, and then finally the sugar is formed by the splitting of the last dextrin. According to MOREAU, in the first stages of saccharification amylodextrin, erythrodextrin, achroodextrin and sugar are formed simultaneously. Other investigators, especially SYNIEWSKI, have recently suggested other views on this subject.[1]

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[1] In regard to the various views on the theories of the saccharification of starch,

The various dextrins have not as yet been separated from each other, nor isolated as chemical individuals. Recently YOUNG[1] has tried their separation by means of neutral salts, especially ammonium sulphate, and MOREAU by the aid of a baryta-alcohol method. We cannot enter into the differences as to the dextrins so separated, and only the characteristic properties and reactions will be given for the dextrins in general.

The dextrins appear as amorphous, white or yellowish-white powders which are readily soluble in water. Their concentrated solutions are viscid and sticky, similar to gum solutions. The dextrins are dextrogyrate. They are insoluble or nearly so in alcohol, and insoluble in ether. Watery solutions of dextrins are not precipitated by basic-lead acetate. Dextrins dissolve cupric hydrate in alkaline liquids, forming a beautiful blue solution, which, as is generally admitted, is reduced by pure dextrins. According to MOREAU pure dextrin has no reducing action. The dextrins are not directly fermentable.

The **vegetable gums** are soluble in water, forming solutions which are viscid but may be filtered. We designate, on the contrary, as **vegetable mucilages** those varieties of gum which do not or only partly dissolve in water, and which swell up therein to a greater or less extent. The natural varieties of gum and mucilage, to which belong several generally known and important substances, such as gum arabic, wood-gum, cherry-gum, salep, and quince mucilage, and probably also the little-studied pectin substances, will not be treated in detail, because of their unimportance from a physiological standpoint.

The Cellulose Group, $(C_6H_{10}O_5)x$.

Cellulose is that carbohydrate, or perhaps more correctly mixture of carbohydrates, which forms the chief constituent of the walls of the plant-cells. This is true for at least the walls of the young cells, while in the walls of the older cells the cellulose is extensively incrusted with a substance called LIGNIN.

The true celluloses are characterized by their great insolubility. They are insoluble in cold or hot water, alcohol, ether, dilute acids, and alkalies. We have only one specific solvent for cellulose, and that is an ammoniacal solution of copper oxide called SCHWEITZER's reagent. The cellulose may be precipitated from this solvent by the addition of acids, and obtained as an amorphous powder after washing with water.

Cellulose is converted into a substance, so-called AMYLOID, which gives a blue coloration with iodine by the action of concentrated sulphuric acid.

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see Musculus and Gruber, Zeitschr. f. physiol. Chem., **2**; Lintner and Düll, Ber. d. d. chem. Gesellsch., **26** and **28**; Bülow, l. c.; Brown and Heron, Journ. of Chem. Soc., 1879; Brown and Morris, *ibid.*, 1885 and 1889; Moreau, Biochem. Centralbl., **3**, 648; Syniewski, Annal. d. Chem. u. Pharm., **309**, and Chem. Centralbl., 1902, **2**.

[1] Journ. of Physiol., **22**, which contains the older researches of Nasse, Krüger, Neumeister, Pohl, and Halliburton. Moreau, l. c.

By the action of strong nitric acid or a mixture of nitric acid and concentrated sulphuric acid celluloses are converted into nitric-acid esters or nitrocelluloses, which are highly explosive and have found great practical use.

The ordinary celluloses when treated at the ordinary temperature with strong sulphuric acid and then boiled for some time after diluting with water are converted into dextrose. We also have celluloses which behave differently, namely, those which yield mannose on the above treatment.

Hemicelluloses are, according to E. SCHULZE, those constituents of the cell-wall related to cellulose which differ from the ordinary cellulose by dissolving on heating with strongly diluted mineral acids, such as 1.25 per cent sulphuric acid, and of yielding arabinose, xylose, galactose, and mannose instead of dextrose. The hemicelluloses (from lupin seeds) are hydrolized even by 0.1 per cent hydrochloric acid and are dissolved, although only slowly, by diastatic enzymes (SCHULZE and CASTORO [1]).

The cellulose, at least in part, undergoes decomposition in the intestinal tract of man and animals. A closer discussion of the nutritive value of cellulose will be given in a future chapter (on digestion). The great importance of the carbohydrates in the animal economy and to animal metabolism will also be given in the following chapters.

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[1] E. Schulze, Zeitschr. f. physiol. Chem., 16 and 19, with Castoro, *ibid.*, 36.

CHAPTER IV.

THE ANIMAL FATS.

THE fats form the third chief group of the organic food of man and animals. They occur very widely distributed in the animal and plant kingdoms. Fat occurs in all organs and tissues of the animal organism, though the quantity may be so variable that a tabular exhibit of the amount of fat in different organs is of little interest. The marrow contains the largest quantity, having over 96 per cent. The three most important deposits of fat in the animal organism are the intermuscular connective tissue, the fatty tissue in the abdominal cavity, and the subcutaneous connective tissues. In plants, the seeds and fruit and in certain instances also the roots, are rich in fat.

The fats consist almost entirely of so-called neutral fats with only very small quantities of fatty acids. The neutral fats are esters of the triatomic alcohol, glycerine, with monobasic fatty acids. These esters are triglycerides, that is, the hydrogen atoms of the three hydroxyl groups of the glycerine are replaced by the fatty-acid radicals, and their general formula is therefore $C_3H_5O_3.R_3$. The animal fats consist chiefly of esters of the three fatty acids, stearic, palmitic, and oleic acids. In certain fats, especially in milk-fat, glycerides of fatty acids such as butyric, caproic, caprylic, and capric acids also occur in considerable amounts. Besides the above-mentioned ordinary fatty acids, stearic, palmitic, and oleic acids, we also find in human and animal fat, exclusive of certain fatty acids only little studied, the following non-volatile fatty acids, as glycerides, namely, lauric acid, $C_{12}H_{24}O_2$, myristic acid, $C_{14}H_{28}O_2$, and arachidic acid, $C_{20}H_{40}O_2$. In the plant kingdom triglycerides of other fatty acids, such as lauric acid, myristic acid, linoleic acid, erucic acid, etc., sometimes occur abundantly. Besides these, oxyacids and high molecular alcohols have been found in many plant fats. The extent to which traces of these oxyacids occur in the animal kingdom has not been thoroughly investigated, but the occurrence of monoxystearic acid seems to have been proven.[1] The occurrence of high molecular alcohols, although ordinarily only in small amounts, has on the contrary been positively shown in animal fat.

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[1] Erben, Zeitschr. f. physiol. Chem., 30; Bernert, Arch. f. exp. Path. u. Pharm., 49.

The animal fats are of the greatest interest and consist of a mixture of varying quantities of TRISTEARIN, TRIPALMITIN, and TRIOLEIN, having an average elementary composition of C 76.5, H 12.0, and O 11.5 per cent. It must be remarked that in animal fat (mutton and beef tallow) as well as in plant fat (olive-oil) mixed triglycerides, such as dipalmityl-olein, distearyl-palmitin and distearyl-olein, occur and that these mixed glycerides may also be prepared synthetically.[1]

Fats from different species of animals, and even from different parts of the same animal, have an essentially different consistency, depending upon the relative amounts of the different individual fats present. In solid fats —as tallow—tristearin and tripalmitin are in excess, while the less solid fats are characterized by a greater abundance of tripalmitin and triolein. This last-mentioned fat is found in greater quantities proportionally in cold-blooded animals, and this accounts for the fact that the fat of these animals remains fluid at temperatures at which the fat of warm-blooded animals solidifies. Human fat from different organs and tissues contains, in full numbers, 67–85 per cent triolein.[2] The melting-point of different fats depends upon the composition of the mixtures, and it not only varies for fat from different tissues of the same animal, but also for the fat from the same tissues in various kinds of animals.

Neutral fats are colorless or yellowish and, when perfectly pure, odorless and tasteless. They are lighter than water, on which they float when in a molten condition. They are insoluble in water, dissolve in boiling alcohol, but separate on cooling—often in crystals. They are easily soluble in ether, benzene, and chloroform. The fluid neutral fats give an emulsion when shaken with a solution of gum or albumin. With water alone they give an emulsion only after vigorous and prolonged shaking, but the emulsion is not persistent. The presence of some soap causes a very fine and permanent emulsion to form easily. Fat produces spots on paper which do not disappear; it is not volatile; it boils at about 300° C. with partial decomposition, and burns with a luminous and smoky flame. The fatty acids have most of the above-mentioned properties in common with the neutral fats, but differ from them in being soluble in alcohol-ether, in having an acid reaction, and by not giving the acrolein test. The neutral fats generate a strong irritating vapor of acrolein, due to the decomposition of glycerine, $C_3H_5(OH)_3-2H_2O=C_3H_4O$, when heated alone, or more easily when heated with potassium bisulphate or with other dehydrating substances.

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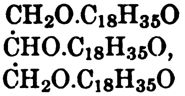
[1] Guth, Zeitschr. f. Biologie, **44**; W. Hansen, Arch. f. Hygiene, **42**; Holde and Stange, Ber. d. d. chem. Gesellsch., **34**; Kreis and Hafner, *ibid.*, **36**.

[2] See Knöpfelmacher, "Untersuch. über das Fett im Säuglingsalter," etc., Jahrbuch f. Kinderheilkunde (N. F.), **45**, which also contains the older literature; Jaeckle, Zeitschr. f. physiol. Chem., **36**.

The neutral fats may be split by the addition of the constituents of water according to the following equation: $C_3H_5(OR)_3 + 3H_2O = C_3H_5(OH)_3 + 3HOR$. This splitting may be produced by the pancreatic enzyme and other enzymes occurring in the animal and vegetable kingdoms, or by superheated steam. We most frequently decompose the neutral fats by boiling them with not too concentrated caustic alkali, or, still better (in biochemical researches), with an alcoholic potash solution or with sodium alcoholate. By this procedure, which is called saponification, the alkali salts of the fatty acids (soaps) are formed. If the saponification is made with lead oxide, then lead plaster, the lead salt of the fatty acids, is produced. By saponification is to be understood not only the cleavage of neutral fats by alkalies, but also the splitting of neutral fats into fatty acids and glycerine in general.

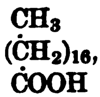
On keeping fats for a long time in contact with air they undergo a change, becoming yellow in color and acid in reaction, and they develop an unpleasant odor and taste, becoming *rancid*. In this change a part of the fat is split into fatty acids and glycerine, and then an oxidation of the free fatty acids takes place, producing volatile bodies of an unpleasant odor.

The three most important fats of the animal kingdom are *stearin, palmitin*, and *olein*.



Stearin or tristearin, $C_{57}H_{110}O_6 = \begin{array}{l} CH_2O.C_{18}H_{35}O \\ \dot{C}HO.C_{18}H_{35}O \\ \dot{C}H_2O.C_{18}H_{35}O \end{array}$, occurs especially in the solid varieties of tallows, but also in the vegetable fats. Stearic acid, $C_{18}H_{36}O_2$, is found in the free state in decomposed pus, in the expectorations in gangrene of the lungs, and in cheesy tuberculous masses. It occurs as lime soap in excrements and adipocere, and in this last product also as an ammonium soap. It also exists as alkali soap in the blood, bile, transudations and pus, and in the urine to a slight extent.

Stearin is the hardest and most insoluble of the three ordinary neutral fats. It is nearly insoluble in cold alcohol, and soluble with great difficulty in cold ether (225 parts). It separates from warm alcohol on cooling as rectangular, less frequently as rhombic plates. The statements in regard to the melting-point are somewhat varied. Pure stearin, according to HEINTZ,[1] melts transitorily at 55° and permanently at 71.5°. The stearin from the fatty tissues (not pure) melts at 63° C.



Stearic acid, $\begin{array}{l} CH_3 \\ \dot{(C}H_2)_{16} \\ \dot{C}OOH \end{array}$, crystallizes (on cooling from boiling alcohol) in

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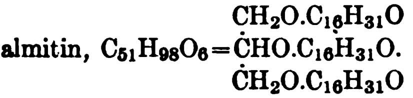
[1] Annal. d. Chem. u. Pharm., 92.

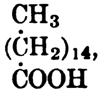
large, shining, long rhombic scales or plates. It is less soluble than the other fatty acids and melts at 69.2° C. Its barium salt contains 19.49 per cent barium, and its silver salt contains 27.59 per cent silver.



$$C_{51}H_{98}O_6 = \begin{array}{l} CH_2O.C_{16}H_{31}O \\ \dot{C}HO.C_{16}H_{31}O \\ \dot{C}H_2O.C_{16}H_{31}O \end{array}$$

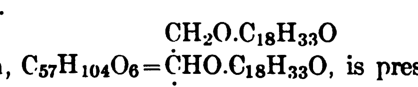
Palmitin, or tripalmitin, $C_{51}H_{98}O_6$ = CHO.$C_{16}H_{31}O$. Of the two solid varieties of fats, palmitin is the one which occurs in predominant quantities in human fat (LANGER[1]). Palmitin is present in all animal fats and in several kinds of vegetable fat. A mixture of stearin and palmitin was formerly called MARGARIN. As to the occurrence of palmitic acid, $C_{16}H_{32}O_2$, about the same remarks apply as to stearic acid. The mixture of these two acids has been called margaric acid, and this mixture occurs—often as very long, thin, crystalline plates—in old pus, in expectorations from gangrene of the lungs, etc.

Palmitin crystallizes, on cooling from a warm saturated solution in ether or alcohol, in starry rosettes of fine needles. The mixture of palmitin and stearin, called margarin, crystallizes, on cooling from a solution, as balls or round masses which consist of short or long, thin plates or needles which often appear like blades of grass. Palmitin, like stearin, has a variable melting and solidifying point, depending upon the way it has been previously treated. The melting-point is often given as 62° C. According to other statements,[2] it melts at 50.5° C., solidifies on further heating, and melts again at 66.5° C.



$$\begin{array}{l} CH_3 \\ (\dot{C}H_2)_{14} \\ \dot{C}OOH \end{array}$$

Palmitic acid, $(CH_2)_{14}$, crystallizes from an alcoholic solution in tufts of fine needles. It melts at 62° C.; still the admixture with stearic acid, as HEINTZ has shown, essentially changes the melting- and solidifying-points according to the relative amounts of the two acids. Palmitic acid is somewhat more soluble in cold alcohol than stearic acid; but they have about the same solubility in boiling alcohol, ether, chloroform, and benzene. Its barium salt contains 21.17 per cent barium, and the silver salt contains 29.72 per cent silver.



$$C_{57}H_{104}O_6 = \begin{array}{l} CH_2O.C_{18}H_{33}O \\ \dot{C}HO.C_{18}H_{33}O \\ \dot{C}H_2O.C_{18}H_{33}O \end{array}$$

Olein, or triolein, $C_{57}H_{104}O_6$ = CHO.$C_{18}H_{33}O$, is present in all animal fats, and in greater quantities in vegetable fats. It is a solvent for stearin and palmitin. The oleic acid (elaic acid), $C_{18}H_{34}O_2$, has as soaps probably about the same occurrence as the other fatty acids.

Olein is, at ordinary temperatures, a nearly colorless oil of a specific gravity of 0.914, without odor or marked taste, and solidifies in crystalline needles at −6° C. It becomes rancid quickly if exposed to the air. It dissolves with difficulty in cold alcohol, but more easily in warm alcohol or in ether. It is converted into its isomer, ELAIDIN, by nitrous acid.

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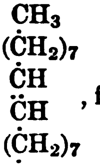
[1] Monatshefte f. Chem., 2; see also Jaeckle, Zeitschr. f. physiol. Chem., 36.

[2] R. Benedikt, Analyse der Fette, 3. Aufl., 1897, p. 44.

Oleic acid, 
$$\begin{array}{l} CH_3 \\ (\dot{C}H_2)_7 \\ \dot{C}H \\ \ddot{C}H \\ (\dot{C}H_2)_7 \\ \dot{C}OOH \end{array}$$
, forms on heating, besides volatile acids, *sebacic acid*, $C_{10}H_{18}O_4$, which crystallizes in shining leaves and melts at 127° C. With nitrous acid oleic acid is transformed into the isomeric solid *elaidic acid*, which melts at 45° C. Oleic acid forms at ordinary temperature a colorless, tasteless, and odorless oily liquid which solidifies in crystals at about 4° C., which then melt again at 14° C. Oleic acid is insoluble in water, but dissolves in alcohol, ether, and chloroform. With concentrated sulphuric acid and some cane-sugar it gives a beautiful red or reddish-violet liquid whose color is similar to that produced in PETTENKOFER'S test for bile-acids. Oleic acid is an unsaturated fatty acid which can take up halogens. On heating with hydriodic acid and amorphous phosphorus it takes up hydrogen and is converted into stearic acid. Oleic acid readily oxidizes in the air, yielding acid products. The monoxystearic acid found in certain animal fats may be formed from oleic acid by oxidation. The barium salt of oleic acid contains 19.65 per cent barium and the silver salt 27.73 per cent silver.

If the watery solution of the alkali compounds of oleic acid is precipitated with lead acetate, a white, tough, sticky mass of lead oleate is obtained which is not soluble in water and only slightly in alcohol, but is soluble in ether. This salt is more easily soluble in benzene than the lead salts of stearic and palmitic acids, and this behavior of the lead salts towards ether and benzene is made use of in separating oleic acid from the other fatty acids.

An acid related to oleic acid, DOEGLIC ACID, which is solid at 0° C., liquid at 16° C., and soluble in alcohol, is found in the blubber of the Balœna rostrata. KURBATOFF [1] has demonstrated the presence of linoleic acid in the fat of the silurus, sturgeon, seal, and certain other animals. Drying fats have also been found by AMTHOR and ZINK [2] in hares, wild rabbits, wild boar, and mountain-cock.

To detect the presence of fat in an animal fluid or tissue the fat must first be shaken out or extracted with ether. After the evaporation of the ether the residue is tested for fat and the acrolein test must not be neglected. If this test gives positive results, then neutral fats are present; if the results are negative, then only fatty acids are present. If the above residue after evaporation gives the acrolein test, then a small portion is dissolved in alcohol-ether free from acid and which has been colored bluish violet by tincture of alkanet. If the color becomes red, a mixture of neutral fat and fatty acids is present. In this case the fat is treated while warm with a soda solution and evaporated on the water-bath, with constant stirring until all the water is removed. The fatty acids hereby combine with the alkali, forming soaps, while the neutral fats are not saponified under these conditions. If this mixture of soaps and neutral fats is treated with water and then shaken with pure ether, the neutral fats are dissolved, while the soaps remain in the watery solution. The fatty acids may be separated from this solution by the addition of a mineral acid which sets the acid free.

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[1] Maly's Jahresber., 22.

[2] Zeitschr. f. analyt. Chem., 36.

The neutral fats separated from the soaps by means of ether are often contaminated with cholesterin, which must be separated in quantitative determinations by saponification with alcoholic caustic potash. The cholesterin is not attacked by the caustic alkali, while the neutral fats are saponified. After the evaporation of the alcohol the residue is dissolved in water and shaken with ether, which dissolves the cholesterin. The fatty acids are separated from the watery solution of the soaps by the addition of a mineral acid. If a mixture of soaps, neutral fats, and fatty acids is originally present, it is treated first with water, then agitated with ether free from alcohol, which dissolves the fat and fatty acids, while the soaps remain in the solution, with the exception of a very small amount which is dissolved by the ether.

To detect and to separate the different varieties of neutral fats from each other it is best first to saponify them with alcoholic potash, or still better with sodium alcoholate, according to KOSSEL, OBERMÜLLER, and KRÜGER.[1] After the evaporation of the alcohol the salts of the fatty acids are dissolved in water and precipitated with sugar of lead. The lead oleate is then separated from the other two lead salts by repeated extraction with ether, but it must be remarked that the lead salts of the other fatty acids are not quite insoluble in ether. The residue insoluble in ether is decomposed on the water-bath with an excess of soda solution, evaporated to dryness, finely pulverized, and extracted with boiling alcohol. The alcoholic solution is then fractionally precipitated by barium acetate or barium chloride. In one fraction the amount of barium is determined, and in the other the melting-point of the fatty acid set free by a mineral acid. The fatty acids occurring originally in the animal tissues or fluids as free acids or as soaps are converted into barium salts and investigated as above. According to JAECKLE,[2] it is better to isolate the fatty acids as silver salts. This same experimenter also considers it more advisable to dissolve the lead salts in warm benzene, as suggested by FARNSTEINER, and to obtain the crystalline lead salts of the solid fatty acids by cooling.

In addition to the methods already suggested there are other chemical methods which are important in investigating fats. Besides ascertaining the melting- and congealing-point we also determine the following: 1. The *acid equivalent*, which is a measure of the amount of fatty acids in a fat and is determined by titrating the fat dissolved in alcohol-ether with N/10 alcoholic caustic potash, using phenolphthalein as indicator. 2. The *saponification equivalent*, which gives the milligrams of caustic potash united with the fatty acids in the saponification of 1 gram fat with N/2 alcoholic caustic potash. 3. Reichert-Meissl's *equivalent*, which gives the quantity of volatile fatty acids contained in a given amount of neutral fat (5 grams). The fat is saponified, then acidified with mineral acid and distilled, whereby the volatile fatty acids pass over and the distillate is titrated with alkali. 4. *Iodine equivalent* is the quantity of iodine absorbed by a certain amount of the fat by addition. It is chiefly a measure of the quantity of unsaturated fatty acids, principally oleic acid or olein, in the fat. Other bodies, such as cholesterin, may also absorb iodine or halogens. The iodine equivalent is generally determined according to the method suggested by v. Hübl. 5. The *acetyl equivalent*. Oxyacids, alcohols such as cetyl alcohol or cholesterin, and those constituents of fats containing the OH group are transformed into the corresponding acetyl ester on boiling with acetic anhydride, while the fatty acids remain unchanged, and in this way the estimation of these bodies is possible. The fat is saponified, the soaps decomposed by an excess of acid, and the mixture of fatty acids, oxyfatty acids, cholesterin, etc., boiled with acetic anhydride. The acid equivalent is determined in a weighed part of the carefully washed acetic-acid-free mixture by titration with alcoholic caustic potash. This acid equivalent represents all the acids (fatty acids as well as the acetylated oxyacids), and it is designated the *acetyl-acid equivalent*. The neutral fluid is now titrated with an exactly measured, sufficient quantity of the same alkali and the acetyl compounds saponified by boiling. On retitrating we find the quantity of alkali used in saponification, and this number, calculated to 100 parts of the fat, represents the acetyl equivalent. In regard to the performance of the above-mentioned different estimations we must refer the reader to more complete works, such as "Analysis of Fats and Waxes," R. Benedikt, 1897.

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[1] Zeitschr. f. physiol. Chem., **14**, **15**, and **16**.

[2] *Ibid.*, **36**.

In the quantitative estimation of fats the finely divided dried tissues or the finely divided residue from an evaporated fluid is extracted with ether, alcohol-ether, benzene, or any other proper extraction medium. The investigations of Dormeyer [1] and others, carried on in Pflüger's laboratory, have shown that even with very prolonged extraction with ether all the fat is not extracted. First extract the greater part of the fat by ether. Then digest with pepsin-hydrochloric acid, collect the insoluble residue on a filter, dry, and extract with ether. The fat is extracted from the filtrate by shaking with ether, evaporating the extract and the fat separated from other bodies by extracting the residue with petroleum ether. Lecithin and other bodies are dissolved by the various solvents, hence the results for the fats may be too high. This is especially the case on using the saponification method [2] suggested by Liebermann and Székely, whereby the lecithins as well as the fats are saponified. Glikin [3] recommends as the best procedure the extraction with boiling petroleum ether and the removal of the lecithin by acetone, in which it is insoluble.

The fats are poor in oxygen, but rich in carbon and hydrogen. They therefore represent a large amount of chemical potential energy, and yield correspondingly large quantities of heat on combustion. They take first rank among the foods in this regard, and are therefore of very great importance in animal life. We will speak more in detail of this significance, also of fat formation and of the behavior of the fats in the body, in the following chapters.

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[1] On fat extraction for quantitative estimation see Dormeyer, Pflüger's Arch., **61** and **65**; Bogdanow, *ibid.*, **65**, **68**, and Arch. f. (Anat. u.) physiol., 1897, 149; N. Schulz, Pflüger's Arch., **66**; Voit and Krummacher, Zeitschr. f. Biologie, **35**; O. Frank, *ibid.*, **35**; Polimanti, Pflüger's Arch., **70**; J. Nerking, *ibid.*, **71**.

[2] Pflüger's Arch., **72**, and Liebermann, *ibid.*, **108**.

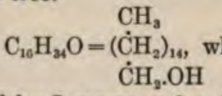
[3] *Ibid.*, **95**.

The LECITHINS, which stand in close relationship to the fats, will be treated in a subsequent chapter (V). The following bodies are related to the ordinary animal fats.

Spermaceti. In the living spermaceti or white whale there is found in a large cavity in the skull an oily liquid called spermaceti, which on cooling after death separates into a solid crystalline part ordinarily called SPERMACETI, and into a liquid, SPERMACETI-OIL. This last is separated by pressure. Spermaceti is also found in other whales and in certain species of dolphin.

The purified, solid spermaceti, which is called CETIN, is a mixture of esters of fatty acids. The chief constituent is the cetyl-palmitic ester mixed with small quantities of compound esters of lauric, myristic, and stearic acids with radicals of the alcohols, LETHAL, $C_{12}H_{25}.OH$, METHAL, $C_{14}H_{29}.OH$, and STÈTHAL, $C_{18}H_{37}.OH$.

Cetin is a snow-white mass shining like mother-of-pearl, crystallizing in plates, brittle, fatty to the touch, and which has a varying melting-point of 30° to 50° C., depending upon its purity. Cetin is insoluble in water, but dissolves easily in cold ether or volatile and fatty oils. It dissolves in boiling alcohol, but crystallizes on cooling. It is saponified with difficulty by a solution of caustic potash in water, but with an alcoholic solution it saponifies readily and the above-mentioned alcohols are set free.



Ethal or cetyl alcohol, $C_{16}H_{34}O = \begin{matrix} CH_3 \\ (\dot{C}H_2)_{14} \\ \dot{C}H_2.OH \end{matrix}$, which occurs in smaller quantities in beeswax, and was found by Ludwig and v. Zeynek[1] in the fat from dermoid cysts, forms white, transparent, odorless, and tasteless crystals which are insoluble in water but dissolve easily in alcohol and ether. Ethal melts at 49.5° C.

Spermaceti-oil yields on saponification valerianic acid, small amounts of solid fatty acids, and PHYSETOLEIC ACID. This acid, which has, like hypogæic acid, the composition $C_{16}H_{30}O_2$, occurs also, as found by Ljubarsky,[2] in considerable amounts in the fat of the seal. It forms colorless and odorless needle-shaped crystals which easily dissolve in alcohol and ether and melt at 34° C.

Beeswax may be treated here as concluding the subject of fats. It contains three chief constituents: (1) CEROTIC ACID, $C_{26}H_{52}O_2$,[3] which occurs as cetyl ether in Chinese wax and as free acid in ordinary wax. It dissolves in boiling alcohol and separates as crystals on cooling. The cooled alcoholic extract of wax contains (2) CEROLEIN, which is probably a mixture of several bodies, and (3) MYRICIN, which forms the chief constituent of that part of wax which is insoluble in warm or cold alcohol. Myricin consists chiefly of palmitic-acid ester of melissyl (myricyl) alcohol, $C_{30}H_{61}.OH$. This alcohol is a silky, shining, crystalline body melting at 85° C.

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[1] Zeitschr. f. physiol. Chem., **23**.

[2] Journ. f. prakt. Chem. (N. F.), **57**.

[3] See Henriques, Ber. d. deutsch. chem. Gesellsch., **30**, 1415.

CHAPTER V.

THE ANIMAL CELL.

THE *cell* is the unit of the manifold variable forms of the organism; it forms the simplest physiological apparatus, and as such is the seat of chemical processes. It is generally admitted that all chemical processes of importance do not take place in the animal fluids, but transpire in the cells, hence the cell may be considered as the chemical laboratory of the organism. It is also principally the cells which, through their greater or less activity, regulate or govern the range of the chemical processes, and also the extensiveness of the total exchange of material.

It is natural that the chemical investigation of the animal cell should in most cases be in reality a study of those tissues of which it forms the chief constituent. Only in a few cases can the cells, by relatively simple manipulations, be directly isolated in a rather pure state from the tissues, as, for example, in the investigation of pus or of tissues very rich in cells. But even in these cases the chemical investigation may not lead to any positive results in regard to the constituents of the uninjured living cells. By the process of chemical transformation new substances may be formed on the death of the cell, and at the same time physiological constituents of the cell may be destroyed or transported into the surrounding medium and therefore escape investigation. For this and other reasons we possess only a very limited knowledge of the constituents and the composition of the cell, especially of the living one.

While young cells of different origin in the early period of their existence may show a certain similarity in regard to form and chemical composition, they may, on further development, not only take the most varied forms, but may also offer from a chemical standpoint the greatest diversity. As a description of the constituents and composition of the different cells occurring in the animal organism is nearly equivalent to a demonstration of the chemical properties of most animal tissues, and as this exposition will be found in the corresponding chapters, we will here discuss only the chemical constituents of the young cells or cells in general.

In the study of these constituents we are confronted with another difficulty, namely, we must differentiate by chemical research between those constituents which are essentially necessary for the life of the cells and those which are casual, i.e., stored up as reserve material or as metabolic products. In this connection we have only been able, thus far, to learn of certain substances which seem to occur in every developing cell. Such bodies, called PRIMARY by KOSSEL,[1] are, besides water and certain mineral constituents, proteins, nucleoproteids or nucleins, lecithins, glycogen (?), and cholesterin. Those bodies which do not occur in every developing cell are called SECONDARY. Among these we have fat, glycogen (?), pigments, etc. It must not be forgotten that it is still possible that other primary cell constituents may exist, as yet unknown to us, and we also do not know whether all the primary constituents of the cell are necessary or essential for the life and functions of the same.

Another important question is the division of the various cell constituents between the two morphological components of the cell, namely, the protoplasm and the nucleus. This is very difficult to decide for many of the constituents; nevertheless it is appropriate to differentiate between the protoplasm and the nucleus.

The Protoplasm of the developing cell consists during life of a semisolid mass, contractile under certain conditions and readily changeable, which is rich in water and whose chief portion consists of protein substances, i.e., of colloids. If the cell be deprived of the physiological conditions of life, or if exposed to destructive exterior influences, such as the action of high temperatures or of chemical agents, the protoplasm dies. The protein bodies which it contains coagulate at least partially, and other chemical changes are found to take place. The alkaline reaction (litmus) of the living cell may become acid by the appearance of paralactic acid, and the carbohydrate, glycogen, which habitually occurs in many cells, may after their death be quickly changed and consumed.

The question as to the internal structure of the protoplasm is still in controversy. It is of little importance in the study of the chemical composition of the cells, as it is impossible to study, especially by chemical means, the morphologically different constituents of the protoplasm. With the exception of a few microchemical reactions the chemical analysis has been restricted to the protoplasm as such, and the investigations have been directed in the first place to the protein substances which form the chief mass of the protoplasm.

The proteins of the protoplasm consist, according to the older general view, chiefly of *globulins*. *Albumins* have also been found besides the globulins. There is no doubt at present that the albumins occur in the cells only as traces, or at least only in trifling quantities. The presence of globulins can hardly be disputed, although certain cell constituents described as globulins have been shown on closer investigation to be nucleoalbumins or nucleoproteids. According to HALLIBURTON[1] the protein occurring in all cells and coagulating at 47–50° C. is a true globulin.

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[1] Verhandl. d. physiol. Gesellsch. zu Berlin, 1890–91 Nos. 5 and 6.

In opposition to the view that the chief mass of the animal cell consists of true proteids, HAMMARSTEN[2] expressed the opinion several years ago that the chief mass of the protein substances of the cells does not consist of proteids in the ordinary sense, but consists of more complex phosphorized bodies, and that the globulins and albumins are to be considered as nutritive material for the cells or as destructive products in the chemical transformation of the protoplasm. This view has received substantial support by investigations within the last few years. ALEX. SCHMIDT[3] has come to the view, by investigations on various kinds of cells, that they contain only very little proteid, and that the chief mass consists of very complex protein substances.

The protein substances of the cells consist chiefly of *compound proteids*, and these are divided between the glucoproteid and the nucleoproteid groups. It is impossible at present to state to what extent nucleoalbumins exist in the cells, because thus far in most cases no exact difference has been made between them and the nucleoproteids. HOPPE-SEYLER[4] calls *vitellin* a regular constituent of all protoplasm. This body used to be considered as a globulin, but later researches have shown that the so-called vitellin bodies may be of various kinds. Certain vitellins seem to be nucleoalbumins, and it is therefore very probable that cells habitually contain *nucleoalbumins*.

The *nucleoproteids* take a very prominent place among the compound proteids of the cell. The various substances isolated by different investigators from animal cells, such as *tissue-fibrinogen* (WOOLDRIDGE), *cytoglobin* and *präglobulin* (ALEX. SCHMIDT), or *nucleohistone* (KOSSEL and LILIENFELD[5]), belong to this group. The cell constituent which swells up to a sticky mass with common salt solution and is called ROVIDA'S *hyaline substance* also belongs to this group.

The above-mentioned different protein substances have simply been designated as constituents of the cells. The next question is which of these belong to the protoplasm and which to the nucleus. At present we can give no positive answer to this question. According to KOSSEL and LILIENFELD,[1] the cell-nucleus of the leucocytes of the thymus gland contains a nucleoproteid as chief constituent, besides nucleins, and sometimes perhaps also nucleic acid (see below), while the body of the cells contains chiefly pure proteids, besides other substances, and a nucleoproteid, containing only a very small quantity of phosphorus. As the lymphocytes of the thymus gland of the calf contain only one nucleus, in which the mass of the nucleus surpasses that of the cytoplasm, it is natural that the relative proportion of the various protein substances in these cells cannot be taken as a standard for the composition of other cells richer in cytoplasm.

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[1] See Halliburton, On the Chemical Physiology of the Animal Cell, 1893, No. 1, King's College Physiol. Laboratory.

[2] Pflüger's Arch., 36, 449.

[3] Alex. Schmidt, Zur Blutlehre, Leipzig, 1892.

[4] Physiol. Chem., 1877–1881, 76.

[5] See L. C. Wooldridge, Die Gerinnung des Blutes, Leipzig, 1891; A. Schmidt, Zur Blutlehre; Lilienfeld, Zeitschr. f. physiol. Chem., 18.

Complete investigations in regard to the distribution of protein substances in the protoplasm and nucleus of other cells have not been made. If we consider for the present that the cells rich in protoplasm contain, as a rule, only very little true proteid, we are hardly wrong in considering it probable that the protoplasm contains chiefly nucleoalbumins and compound proteids besides traces of albumin and a little globulin. These compound proteids are in certain cases glucoproteids, but otherwise nucleoproteids, which differ from the nucleoproteids of the nucleus in being poorer in phosphorus, besides containing a great deal of proteid and only a little of the prosthetic group, and hence have no specially pronounced acid character.

The nucleoproteids of the nucleus are on the contrary, as shown by LILIENFELD and KOSSEL, rich in phosphorus and of a strongly acid character. These nucleoproteids will be treated in speaking of the nucleic acids of the nucleus.

In cases in which the protoplasm is surrounded by an outer, condensed layer or a cell membrane, this envelope seems to consist of albuminoid substances. In a few cases these substances seem to be closely related to elastin; in other cases, on the contrary, they seem rather to belong to the keratin group. Even in cells which do not seem to have any visible special layers forming boundaries, we still admit of such layers on account of the behavior of the cells as regards permeability.

NERNST[2] has shown by a special experiment that the permeability of a membrane for a certain substance is essentially dependent upon the solvent power of the membrane for the said substance. This point, which is of the greatest importance in the study of osmotic phenomena in living cells, has been specially investigated by OVERTON.[3] The behavior of the living cells towards dyestuffs, also the ready introduction into animal and plant protoplasm of such bodies as are insoluble or only slightly soluble in water but readily soluble in fats or fat-like bodies, has led OVERTON to conclude that the protoplasm-boundary layer behaves like a substance layer whose solvent power is closely related to the fatty oils. According to this investigator, the protoplasm-boundary layer is probably impregnated with lipoids, i.e., with lecithins, cholesterin, and bodies similar to protagon, and among which lecithin, which also takes up water, must be of the greatest importance.

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[1] Ueber die Wahlverwandtschaft der Zellelemente zu gewissen Farbstoffen, Verhandl. d. physiol. Gesellsch. zu Berlin, No. 11, 1893.

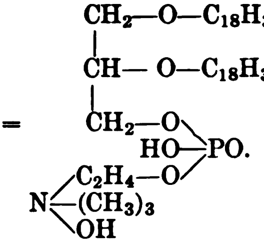
[2] Zeitschr. f. physikal. Chem., 6.

[3] Vierteljahrsschr. d. Naturf. Ges. in Zurich, 44 (1899), and Overton, Studien über die Narkose, Jena, 1901.

The cholesterins and the protagons will be best treated in another connection (see Chapters VIII and XII). We will discuss here only the lecithins, which are present in every cell.

Lecithins. These bodies are ester compounds[1] of glycerophosphoric acid substituted by two fatty-acid radicals with a base called choline. According to the kind of fatty acid contained in the lecithin molecule it is possible to have various lecithins, such as stearyl-, palmityl-, and oleyl-lecithins. According to THUDICHUM[2] two different fatty acids may exist simultaneously in one lecithin, and according to him every true lecithin always contains at least one oleic-acid radical. All lecithins are mononitrogenous monophosphatides, which contain 1 atom of nitrogen for every atom of phosphorus. As an example of a lecithin we give the one closely studied by HOPPE-SEYLER and DIACONOW,[3] called distearyl-lecithin,

$C_{44}H_{90}NPO_9 =$



According to HENRIQUES and HANSEN[4] the iodine equivalent of the fluid fatty acids obtained from egg as well as brain lecithin is higher than that of oleic acid, hence it follows that the lecithins contain other fatty acids besides stearic, palmitic, and oleic acids.

ERLANDSEN[5] in a specially thorough and careful investigation has studied the phosphatides of the ox heart and ox muscles. The lecithin had the same composition as that from the egg-yolk. The iodine equivalent as well as the analysis show that the fatty acids occurring in the lecithin molecule are very poor in hydrogen and belong in part to the linolic or linolenic acid series. Diaminomonophosphatides, i.e., compounds in which the relationship N:P is not, as in lecithin, 1:1, but 2:1, occur in the muscles, but chiefly in the heart muscle. These phosphatides are isolated as metallic salts, and the cadmium compound of the diaminomonophosphatide obtained from the heart had the composition $C_{40}H_{75}N_2PO_{12}.2CdCl_2$. Erlandsen has isolated a new phosphatide from the heart, which he calls *cuorin* and which belongs to the group of monaminodiphosphatides, in which the relation of N:P is 1:2. This cuorin, which occurs only in traces in other muscles, contains two phosphoric-acid radicals which in part are united with glyceryl. Besides these it contains two residues of strongly unsaturated fatty acids and a basic radical, which is not identical with choline. The empirical formula is $C_{71}H_{125}NP_2O_{21}$. Cuorin is soluble in ether but insoluble in alcohol, and is characterized by a very great autooxidizability. It is obtained in the amorphous state. The monaminophosphatides (lecithin and cuorin) can be directly extracted from the air-dried and finely divided organs, and to all appearances occur in the free state. The diaminophosphatides are also soluble in ether, but cannot be directly extracted by ether, but only after a previous treatment with alcohol, and therefore probably exist in combination with proteins.

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[1] Strecker, Annal. d. Chem. u. Pharm., 148; Hundeshagen, Journ. f. prakt. Chem. (N. F.), 28; Gilson, Zeitschr. f. physiol. Chem., 12.

[2] J. L. W. Thudichum, Die chemische Konstitution des Gehirns des Menschen, etc., Tübingen, 1901.

[3] Hoppe-Seyler, Med. chem. Untersuch., Heft 2 and 3.

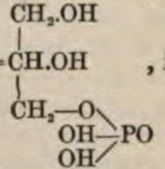
[4] Skand. Arch. f. Physiol., 14.

[5] A. W. E. Erlandsen, Undersögelser over Hjertets Phosphatider, Copenhagen, 1906.

Winterstein and Hiestand,[1] and previous to them Schulze and Winterstein, have isolated from different parts of plants, lecithin preparations which are poorer in phosphorus than the ordinary lecithin, containing as a maximum 2.74 per cent phosphorus, and which on cleavage with dilute mineral acids yielded, besides fatty acids, glycerophosphoric acid, and choline, also considerable quantities of hexoses, indeed 16 per cent. The hexoses were *d*-glucose and *d*-galactose, and besides these small quantities of pentoses were found. These phosphatides seem to be widely distributed in the plant kingdom.

On saponification with alkalies or baryta-water, lecithin yields fatty acids, glycerophosphoric acid, and choline. It is only slowly decomposed by dilute acids. Besides small quantities of glycerophosphoric acid we have large quantities of free phosphoric acid split off.

Glycerophosphoric acid, $C_3H_9PO_6 =$



$$\begin{array}{l} CH_2.OH \\ | \\ CH.OH \\ | \\ CH_2{-}O \searrow \\ \quad OH{-}\!\!\rightarrow PO \\ \quad OH \nearrow \end{array}$$

, is a bibasic acid which probably occurs in the animal fluids and tissues only as a cleavage product of lecithins. According to Willstätter and Lüdecke [2] the glycerophosphoric acid split off from lecithins is optically active. Its barium and potassium salts are levorotatory and behave in certain regards differently from the corresponding salts of synthetically prepared glycerophosphoric acid.

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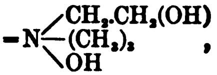
[1] Zeitschr. f. physiol. Chem., **47**.

[2] Willstätter and Lüdecke, Ber. d. d. chem. Gesellsch., **37**.

Choline (trimethyloxyethylammonium hydroxide), $C_5H_{15}NO_2$ =



$$N\begin{cases}CH_2.CH_2(OH)\\(CH_3)_3\\OH\end{cases},$$

which occurs extensively in the plant kingdom, is not identical with the base, NEURINE, prepared by Liebreich as a decomposition product from the brain, which is considered as trimethylvinylammonium hydroxide, $C_5H_{13}NO$. Choline is a syrupy fluid readily miscible with absolute alcohol. Hydrochloric acid gives a compound which is very soluble in water and alcohol, but insoluble in ether, chloroform, and benzene. This compound forms a double combination with platinum chloride which is soluble in water, insoluble in absolute alcohol and ether, crystallizing ordinarily in six-sided orange-colored plates. This compound is used in the detection and identification of this base. Choline also forms a crystalline double compound with mercuric chloride and with gold chloride. Choline is precipitated by potassium iodide and iodine (Gulewitsch), and potassium triiodide can be used for the quantitative estimation of this base (Stanek [1]). On heating the free base it decomposes into trimethylamine, ethylene oxide, and water.

Lecithin occurs, as Hoppe-Seyler [2] has especially shown, widely diffused in the vegetable and animal kingdoms. According to this investigator it occurs also in many cases in loose combination with other bodies, such as proteins, hæmoglobin, and others. Lecithin, according to Hoppe-Seyler, is found in nearly all animal and vegetable cells thus far studied, and also in nearly all animal fluids. It is especially abundant in the brain, nerves, fish eggs, yolk of the egg, electrical organs of the Torpedo electricus, semen, and pus, and also in the muscles and blood-corpuscles, blood-plasma, lymph, milk, especially woman's milk, and bile. Lecithin is also found in different pathological tissues or liquids.

Siwertzow [3] has determined the amount of lecithin in the human fœtus and in children of various ages, and he finds that the quantity of lecithin is much greater in the organs (brain, liver, heart, and muscles) of the ripe fœtus as compared with the same organs of children up to ten years of age. The child according to him has a certain store of lecithin when it comes into the world and this is consumed during the first months of its extra-uterine life.

This wide distribution of the lecithins, as also the fact that they are primary cell constituents, gives great physiological importance to these substances. We have in lecithin, no doubt, a very important material for the building up of the complicated phosphorized nuclein substances of the cell and cell nucleus. That the lecithins are of great importance in the development and growth of living organisms, in fact for the bioplastic processes in general, follows also from several investigations.[1] The fact must not be overlooked that in the animal body we find besides the lecithins also other related phosphatides which have been little studied and which can be readily mistaken for lecithins.

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[1] In regard to choline and its compounds see Gulewitsch, Zeitschr. f. physiol. Chem., 24; Stanek, *ibid.*, 46.

[2] Physiol. Chemie, Berlin, 1877–1881, 57.

[3] See Biochem. Centralbl., 2, 310.

Lecithin may be obtained in grains or warty masses composed of small crystalline plates by strongly cooling its solution in strong alcohol. In the dry state it has a waxy appearance, is plastic, but forms pulverizable masses when dried in vacuum, and is soluble in alcohol, especially on heating (to 40–50° C.); it is less soluble in ether. It is dissolved also by chloroform, carbon disulphide, benzene, and fatty oils. The solution of lecithin from egg-yolk is dextrorotatory (Ulpiani[2]). The solution of lecithin in alcohol-ether or chloroform is precipitated by acetone. It swells in water to a pasty mass which shows under the microscope slimy, oily drops and threads, so-called myelin forms (see Chapter XII). On warming this swollen mass or the concentrated alcoholic solution, decomposition takes place with the production of a brown color. On allowing the solution or the swollen mass to stand, decomposition takes place and the reaction becomes acid.

With considerable water, lecithins give an emulsion or indeed a filterable colloidal solution, which is precipitated by salts with divalent cations, such as Ca, Mg, and others (W. Koch). This precipitate dissolves again in water after the removal from the solution of the electrolytes, and the formation of this precipitate can be prevented by the presence of salts of monovalent cations. We are here not dealing with a chemical but rather with a physical precipitation reaction (Koch[3]). In putrefaction lecithins yield glycerophosphoric acid and choline; the latter further decomposes with the formation of methylamine, ammonia, carbon dioxide, and marsh-gas (Hasebroek[4]). If dry lecithin be heated it decomposes, takes fire, and burns, leaving a phosphorized ash. On fusing with caustic alkali and saltpetre it yields alkali phosphates. Lecithins are easily carried down during the precipitation of other compounds such as the protein bodies, and may therefore very greatly change the solubilities of the latter.

Lecithins combine with acids and bases. The compound with hydrochloric acid give with platinum chloride a double salt which is insoluble in alcohol, soluble in ether, and which contains 10.2 per cent platinum (for distearyl-lecithin). The cadmium-chloride compound which contains

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[1] See Stoklasa, Ber. d. deutsch. chem. Gesellsch., **29**; Wiener Sitzungsber., **104**; Zeitschr. f. physiol. Chem., **25**; W. Danilewsky, Comp. rend., **121** and **123**, and W Koch, Zeitschr. f. physiol. Chem., **37**; P. Kyes, *ibid.*, **41**, and Berl. klin. Wochenschr., 1904.

[2] Chem. Centralbl., 1901, **2**, 30 and 193.

[3] Zeitschr. f. physiol. Chem., **37**.

[4] *Ibid.*, **12**.

3 molecules of lecithin and 4 molecules of cadmium chloride (ULPIANI[1]) is difficultly soluble in alcohol, but dissolves in a mixture of carbon disulphide and ether or alcohol. A solution of lecithins in alcohol is not precipitated by lead acetate and ammonia.

Lecithin may be prepared tolerably pure from the yolk of the hen's egg by the following methods, as suggested by HOPPE-SEYLER and DIACONOW. The yolk, deprived of protein, is extracted with cold ether until all the yellow color is removed. Then the residue is extracted with alcohol at 50-60° C. After the evaporation of the alcoholic extract at 50-60° C., the syrupy matter is treated with ether and the insoluble residue dissolved in as little alcohol as possible. On cooling this filtered alcoholic solution to $-5°$ to $-10°$ C. the lecithin gradually separates in small granules. The ether, however, contains considerable of the lecithin. The ether is distilled off and the residue dissolved in chloroform and the lecithin precipitated from this solution by means of acetone (ALTMANN).

According to GILSON[2] a new portion of lecithin may be obtained from the ether used in extracting the yolk by dissolving the residue after the evaporation of the ether in petroleum-ether and then shaking this solution with alcohol. The petroleum-ether takes the fat, while the lecithin remains dissolved in the alcohol and may be obtained therefrom rather easily by using the proper precautions, as described in the original publication.

ZUELZER'S method is based upon the precipitability of the lecithin by acetone, and BERGELL'S[3] method upon the preparation of the double salt of cadmium and its decomposition by ammonium carbonate. The preparations obtained by the different methods consist generally of a mixture of lecithins.

The detection and the quantitative estimation of lecithins in animal fluids or tissues is based on the solubility of the lecithins (at 50-60° C.) in alcohol-ether, by which the phosphoric-acid or glycerophosphoric-acid salts which may be present at the same time are not dissolved. The alcohol-ether extract is evaporated, the residue dried and fused with soda and saltpetre. Phosphoric acid is formed from the lecithin, and it can be used in the detection and quantitative estimation. The distearyl-lecithin yields 8.798 per cent P_2O_5. This method is, however, not exactly correct, for it is possible that other phosphorized organic combinations, such as jecorin (see Chapter VIII) and protagon (Chapter XII), may have passed into the alcohol-ether extract. In detecting lecithin the double compound of choline and platinum chloride must also be prepared. The residue of the evaporated alcohol-ether extract may be boiled for an hour with baryta-water, filtered, the excess of barium precipitated with CO_2, and filtered while hot. The filtrate is concentrated to a syrupy consistency, extracted with absolute alcohol, and the filtrate precipitated with an alcoholic solution of platinum chloride. The precipitate after filtration may be dissolved in water and allowed to crystallize over sulphuric acid. For the detection and estimation of lecithin we can make use of the method of heating with hydriodic acid as suggested by KOCH.[1] One methyl iodide group is split off at 240° and the two others at about 300° C.

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[1] Chem. Centralbl., 1901, 2, 30 and 193.

[2] Altmann, cited from Hoppe-Seyler-Thierfelder's Handbuch, 7. Auflage; Gilson, ibid.

[3] Zuelzer, Zeitschr. f. physiol. Chem., 27, and Bergell, Ber. d. d. chem. Gesellsch., 33.

Protagons, which are found in the leucocytes and pus-cells, are also to be considered as constituents of protoplasm. These phosphorized bodies occur principally in the brain and nerves, and hence will be described in a following chapter (XII).

Glycogen, first discovered by CL. BERNARD, is found in developing animal cells and especially in developing embryonic tissues. According to HOPPE-SEYLER it seems to be a never-failing constituent of the cells which show amœboid movement, and he found this carbohydrate in the leucocytes, but not in the developed motionless pus-corpuscles. SALOMON and afterwards others have, however, found glycogen in pus.[2] From the relationship which seems to exist between glycogen and muscular work (see Chapter XI), it is presumable that a consumption of glycogen takes place in the movement of animal protoplasm. On the other hand, the extensive occurrence of glycogen in embryonic tissues, as also its occurrence in pathological tumors and in abundant cell formation, speaks for the importance of this body in the formation and development of the cell.

In adult animals glycogen occurs as stored foodstuff in the muscles and certain other organs, but principally in the liver; therefore it will be completely described in connection with this organ (Chapter VIII).

Another body or perhaps more correctly a group of bodies which occur widely distributed in the animal and vegetable kingdoms, and which are present regularly in the cells, are the cholesterins. The best-known representative of this group is ordinary *cholesterin* (see Chapter VIII), which is the chief constituent of certain biliary calculi and exists in abundant quantities in the brain and nerves. It is hardly probable that this body is of direct importance for the life and development of the cell. It must be considered that the cholesterin, as accepted by HOPPE-SEYLER,[3] is a cleavage product appearing in the cell during the processes of life, but this does not exclude the possibility that the cholesterin, as a constituent of the lipoids of the protoplasm-boundary layers (OVERTON), may be of indirect importance in cell life. According to HOPPE-SEYLER, the same is true for the fats, which do not occur constantly in the cells and have nothing to do in the ordinary processes of life. There is no doubt that cholesterin exists as a constituent of the protoplasm, but its existence in the nucleus is questionable. The *intracellular enzymes* are undoubtedly constituents of the protoplasm as well as of the nucleus and must be of the greatest importance for the life and functions of the cells.

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[1] Zeitschr. f. physiol. Chem., **36**, and Amer. Jour. Physiol., **11**.

[2] In regard to the literature on glycogen see Chapter VIII.

[3] Physiol. Chem., p. 81.

The cell nucleus has a rather complex structure. It consists in part of fibrils which form a network and another part which is less solid and homogeneous. The first differs from the second in possessing a stronger affinity for many dyes. On account of this behavior the first is called the chromatic substance or *chromatin,* and the other the achromatic substance or *achromatin.*

The homogeneous substance of the nucleus is considered as a mixture of protein. The network seems to contain the more specific constituent of the nucleus, namely, the nuclein substances. Besides this it is alleged to contain another substance also, *plastin.* This last is less soluble than the nuclein substances and does not have the property, like them, of fixing dyes.

The chief constituents of the cell nucleus are the *nucleoproteids,* and in certain cases the *nucleic acids.*

Nucleoproteids. The most important of these bodies have already been discussed in a previous chapter (II, page 71). These bodies are either strong or loose combinations of nucleic acids with proteid. To the latter class belongs histone, in certain cases, and the compounds between nucleic acids and protamines should also perhaps be called nucleoproteids. There is a difference among the nucleoproteids, dependent on the various proteid complexes as well as upon the nucleic acids. They contain generally considerable proteid in the molecule, hence they give the ordinary proteid reactions, and therefore are closely related to the protein bodies. The nucleoproteids occurring in the cell nucleus seem to be characterized by containing a relatively large amount of phosphorus and a pronounced acid character.

In the preceding discussion of the nucleoproteids, attention was called to the fact that, on their modification by heat, by weak acid action, and by peptic digestion, proteid is split off and a nucleoproteid richer in phosphorus is formed. These compound proteids, rich in nucleic acid, obtained by peptic digestion from cells, cell-rich organs, or nucleoproteids, have been called *nucleins* (MIESCHER, HOPPE-SEYLER[1]) or true nucleins. But as the true nuclein seems to be nothing but a modified nucleoproteid poor in proteid, it seems unnecessary to give the name nuclein thereto. On the other hand, the nucleins have other properties than the nucleoproteids, and as the nucleins bear the same relationship to the nucleoproteids that the pseudonuclein does to the nucleoalbumins, we will give here a short description of the nucleins as well as the pseudo- or paranucleins.

Nucleins or true nucleins are formed, as above stated, from nucleoproteids in their peptic digestion or by treatment with dilute acids. It must be remarked that the nucleins are not entirely resistant towards gastric juice, and also that at least one nucleoproteid, namely, the one obtained from the pancreas, completely dissolves, leaving no nuclein residue on treatment with gastric juice (UMBER, MILROY[1]). The nucleins are rich in phosphorus, containing in the neighborhood of 5 per cent. According to LIEBERMANN,[2] metaphosphoric acid can be split off from true nucleins (yeast nuclein). The nucleins are decomposed into proteid and nucleic acid by caustic alkali, and as different nucleic acids exist, so also there exist different nucleins. As previously stated, proteids may be precipitated in acid solutions by nucleic acids, and in this way, as shown by MILROY, combinations of nucleic acid and proteids may be prepared which behave quite like true nucleins. All nucleins yield so-called *nuclein bases* on boiling with dilute acids. The nucleins contain iron to a considerable extent. They act like rather strong acids.

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[1] Hoppe-Seyler, Med. chem. Untersuch., 452.

The nucleins are colorless, amorphous, insoluble, or only slightly soluble in water. They are insoluble in alcohol and ether. They are more or less readily dissolved by dilute alkalies. The nucleins give the biuret test and MILLON's reaction. They show a great affinity for many dyes, especially the basic ones, and take these up with avidity from watery or alcoholic solutions. On burning they yield an acid residue which is very difficult to incinerate and which contains metaphosphoric acid. On fusion with saltpetre and soda the nucleins yield alkali phosphates.

To prepare nucleins from cells or tissues, first remove the chief mass of proteids by artificial digestion with pepsin-hydrochloric acid, lixiviate the residue with very dilute ammonia, filter, and precipitate with hydrochloric acid. The precipitate is further digested with gastric juice, washed and purified by alternately dissolving in very faintly alkaline water and re-precipitating with an acid, washing with water, and treating with alcohol-ether. A nuclein may be prepared more simply by the digestion of a nucleoproteid. In the detection of nucleins we make use of the above-described method, testing for phosphorus in the product after fusing with saltpetre and soda. Naturally the phosphates, lecithins (and jecorin) must first be removed by treatment with acid, alcohol, and ether, respectively. We must specially call attention to the fact, as shown by LIEBERMANN,[3] that it is very difficult to remove lecithin by means of alcohol-ether. No exact methods are known for the quantitative estimation of nucleins in organs or tissues.

Pseudonucleins or PARANUCLEINS. These bodies are obtained as an insoluble residue on the digestion of certain nucleoalbumins or phosphoglucoproteids with pepsin-hydrochloric acid. Attention is called to the fact that the pseudonuclein may be dissolved by the presence of too much acid or by a too energetic peptic digestion. If the relationship between the degree of acidity and the quantity of substance is not properly selected, the formation of pseudonucleins may be entirely overlooked in the digestion of certain nucleoalbumins. Pseudonucleins contain phosphorus, which, as shown by LIEBERMANN,[1] is split off as metaphosphoric acid by mineral acids.

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[1] Umber, Zeitschr. f. klin. Med., 43; Milroy, Zeitschr. f. physiol. Chem., 22.

[2] Pflüger's Arch., 47.

[3] *Ibid.*

The pseudonucleins are amorphous bodies insoluble in water, alcohol, and ether, but readily soluble in dilute alkalies. They are not soluble in very dilute acids, and may be precipitated from their solution in dilute alkalies by adding acid. They give the protein reactions very strongly, but do not yield nuclein bases.

In preparing a pseudonuclein, dissolve the mother-substance in hydrochloric acid of 1–2 p. m., filter if necessary, add pepsin solution, and allow the mixture to stand at the temperature of the body for about twenty-four hours. The precipitate is filtered off, washed with water, and purified by alternately dissolving in very faintly alkaline water and reprecipitating with acid.

Plastin. After the extraction of the nucleins from cell nuclei of certain plants by dilute soda solution, a residue is obtained which is characterized by its great insolubility. The substance which forms this residue has been called plastin. This substance, of which the spongioplasm of the body of the cell and the nucleus granules are alleged to be composed, is considered as a nuclein modification of great insolubility, although its nature is not known.

Nucleic Acids. All nucleic acids are rich in phosphorus and yield phosphoric acid and nuclein bases as cleavage products. The various nucleic acids are nevertheless very different in regard to the products they yield. The statements in this regard are somewhat contradictory and it seems as if in certain cases we were dealing with impure or partly decomposed nucleic acids. For example, according to KOSSEL, the nucleic acid from ox-sperm yields chiefly xanthine, while LEVENE obtained only guanine and adenine. The guanylic acid isolated by BANG from the pancreas contained only guanine, while the pancreas nucleic acid investigated by LEVENE contained adenine as well as guanine. The nucleic acids of the thymus yield, according to most statements, only adenine and guanine, similar to the acids obtained from the spleen, brain, mammary gland, and fish-sperm. According to STEUDEL, the thymusnucleic acids yield xanthine, hypoxanthine, adenine, and guanine, while according to BANG the thymus gland contains two different nucleic acids, one containing adenine and guanine, while the other contains only adenine, hence is an adenylic acid. The nucleic acid of the intestine yields, according to INOUYE and KOTAKE, all four nuclein bases, although it has about the same composition as the salmonucleic acid, which yields only adenine and guanine.

All nucleic acids thus far investigated, with the exception of guanylic acid, contain also representatives of the pyrimidine group; there seems to exist a difference in this regard between animal and plant nucleic acids. As far as known, in the plant nucleic acids the pyrimidine group is represented only by cytosine and uracil (Kossel, Ascoli, Kossel and Steudel, Osborne and Harris), and in the animal (the thymusnucleic acids), on the contrary, by cytosine, thymine, and uracil (Kossel, Neumann, Levene). Mandel and Levene[1] have nevertheless isolated a nucleic acid from haddock eggs which yielded uracil but no thymine, and this acid behaved in other respects like a nucleic acid from plant-cells. The guanylic acid contains, as above remarked, neither uracil, thymine, nor cytosine.

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[1] Ber. d. d. chem. Gesellsch., 21, and Centralbl. f. d. med. Wissensch., 1889.

The nucleic acids show a different composition also in other regards. A reducing pentose group can be split off from guanylic acid and the vegetable nucleic acids (the tritico- and yeast nucleic acid), while from the yeast nucleic acid also a hexose is claimed to be obtained. No reducing carbohydrate has, on the contrary, been split off from most animal nucleic acids. Certain observations which were based upon qualitative pentose reactions seem to show that the various organs contain nucleoproteids containing pentoses and that we have several nucleic acids which yield pentose (see Chapter III, p. 110). The preparation of these acids in a pure form has been attempted only in a few cases, and the qualitative pentose reactions are not to be relied upon to any great extent. Bang[2] has indeed shown that a nucleic acid occurs in the thymus gland which gives the phloroglucin reaction but does not contain any pentose. Those nucleic acids which do not split off any reducing carbohydrate contain nevertheless a carbohydrate group which, as Kossel and Neumann first showed, on deep cleavage with a mineral acid yields levulinic acid.

We generally admit of 4 atoms of phosphorus in the empirical formulæ of the various nucleic acids. In salmonucleic acid the relationship of phosphorus to nitrogen is as 4 to 14, in triticonucleic acid 4 to 16, and in guanylic acid 4 to 20. The form of combination of the phosphorus is not known with positiveness, but it seems at least that guanylic and triticonucleic acids are derivatives of a pentahydroxylphosphoric acid, $P(OH)_5$.

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[1] Journ. of Biol. Chem., **1**, 425, and Zeitschr. f. physiol. Chem., **49**, 262.

[2] The works of Kossel and his pupils on nucleic acids are found in Arch. f. (Anat. u.) Physiol., 1892, 1893, and 1894; Sitzungsber. d. Berl. Akad. d. Wissensch., **18**, 1894; Centralbl. f. d. med. Wissensch., 1893; Ber. d. deutsch. chem. Gesellsch., **26** and **27**; Zeitschr. f. physiol. Chem., **22** and **38**. See also Neumann, Arch. f. (Anat. u.) Physiol., 1898 and 1899, Suppl.; Miescher, Hoppe-Seyler's Med. chem. Untersuch., 441, and Arch. f. exp. Path. u. Pharm., **37**; Schmiedeberg, *ibid.*, **37** and **43**; Osborne and Harris, Zeitschr. f. physiol. Chem., **36**; Bang, *ibid.*, **26** and **31**; Hofmeister's Beiträge, **5**, and Biochem. Centralbl., **1**, 295; Altmann, Arch. f. (Anat. u.) Physiol., 1899; Ascoli, Zeitschr. f. physiol. Chem., **28** and **31**; Levene, *ibid.*, **32**, **37**, **38**, **39**, **43**, and **45**; Mandel and Levene, *ibid.*, **46**, **47**, **49**, **50**; Inouye and Kotake, *ibid.*, **46**; Steudel, *ibid.*, **42**, **43**, **46**, and **49**.

All nucleic acids are amorphous, white, and have an acid reaction. They are readily soluble in ammoniacal or alkaline water and form insoluble salts with the heavy metals, and as a rule also insoluble basic salts with the alkaline earths. Guanylic acid is soluble with difficulty in cold water but rather readily in boiling water, from which it separates on cooling. Guanylic acid is readily precipitated from its alkali compound by an excess of acetic acid. The other nucleic acids are, on the contrary, not precipitated from such compounds by an excess of acetic acid, but by a slight excess of hydrochloric acid, especially in the presence of alcohol. In acid solutions these latter nucleic acids give precipitates with proteids, which are considered as nucleins. The behavior of guanylic acid in this regard has not been shown on account of the great difficulty in dissolving this acid in dilute acids. All nucleic acids are insoluble in alcohol and ether. They do not give either the biuret test or MILLON'S reaction. The nucleic acids are optically active and indeed dextrorotatory (GAMGEE and JONES[1]).

The proteolytic enzymes, such as pepsin and trypsin, decompose the nucleoproteids more or less; the nucleic acids are not split by these enzymes as far as phosphoric acid and purine bases. Such a cleavage can, on the contrary, be brought about by erepsin (NAKAYAMA) or by other closely allied enzymes which have been called *nucleases* (IWANOFF, FR. SACHS). Micro-organisms can also bring about a more or less deep cleavage of the nucleic acids (SCHITTENHELM and SCHRÖTER[2]).

Guanylic acid differs essentially from the other animal nucleic acids. These latter are closely related to each other, and as they all yield thymine on cleavage and in this regard differ markedly from the guanylic acid and the plant nucleic acids,[3] they can for the present be treated of as one group which has received the common name of thymonucleic acids.

Thymonucleic Acids. A. NEUMANN has isolated α- and β-thymusnucleic acids from the thymus gland. The α-acid is soluble with difficulty and can, according to KOSTYTSCHEW, be transformed (two thirds), with the splitting off of purine bases, into the β-acid. The α-acid gives in proper concentration a sodium salt which gelatinizes and a barium salt which is precipitated by barium acetate in substance (KOSTYTSCHEW). The barium salt of the β-acid is not precipitated by barium acetate. According to BANG, the thymus contains both an adenylic acid and a nucleic acid which contains adenine as well as guanine. This last acid is probably the thymusnucleic acid which is identical with the nucleic acid from the salmon milt (or salmonucleic acid) (SCHMIEDEBERG and HERLANT[1]).

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[1] Proceed. Roy. Soc., 72.

[2] Nakayama, Zeitschr. f. physiol. Chem., 41; Iwanoff, *ibid.*, 39; Fr. Sachs, "Ist die Nuklease mit dem Trypsin identisch?" Inaug.-Dissert. Heidelberg, 1905; Schittenhelm and Schröter, Zeitschr. f. physiol. Chem., 41.

[3] See Mandel and Levene, Jour. of Biol. Chem., 1, 425, and Zeitschr. f. physiol. Chem., 49.

The salmonucleic acid and the thymusnucleic acid as obtained by SCHMIEDEBERG's method have the same composition, $C_{40}H_{56}N_{14}O_{16}.2P_2O_5$. Other nucleic acids, such as those prepared by ALSBERG from the sperm of the burbot (Lota vulgaris), and by LEVENE from ox sperm, brain, and spleen, are identical with the thymusnucleic acid or are at least closely related acids. To this group belong also the nucleic acids from the kidneys and the mammary glands (MANDEL and LEVENE), from the intestinal mucosa (INOUYE and KOTAKE), from the sperm of the sturgeon (NOLL), herring (MATHEWS, GULEWITSCH), and sea-urchin (MATHEWS[2]).

On the decomposition of thymusnucleic acids (or salmonucleic acids) intermediate products of various kinds are produced by a more or less complete cleavage of the nuclein bases. One of these is *thymic acid*, which is obtained on heating the free acid with water at the water-bath temperature, when adenine and guanine are simultaneously split off. Thymic acid is readily soluble in water and yields a barium salt which is also soluble in water and has the formula $C_{16}H_{23}N_3P_2O_{12}Ba$ (KOSSEL and NEUMANN).

On cleavage with acids first a part of the nuclein bases is split off. The remaining part is more difficult to set free, and in this operation an abundant formation of melanin and a decomposition of the original substance take place at the same time. When one half of the purine bases have been split off we obtain the substance called *heminucleic acid* by ALSBERG, which contains only 1 molecule of purine bases to 2 P_2O_5. According to SCHMIEDEBERG, thymusnucleic acid (or salmonucleic acid) is a combination of purine bases with another substance, the *nucleotinphosphoric acid*, $C_{30}H_{46}N_4O_{15}.2P_2O_5$. The non-phosphorized component of this substance, the *nucleotin*, $C_{30}H_{42}N_4O_{13}$, which is the ground substance of thymusnucleic acid, has been isolated by ALSBERG. On the decomposition of nucleic acids with 5 per cent sulphuric acid, LEVENE was able to split off the purine bases completely and the pyrimidine bases in part. The carbohydrate groups went completely into solution.

KUTSCHER and SEEMANN obtained guanidine and urea, but no uric acid, as products on the oxidation of nucleic acid with potassium permanganate. KUTSCHER and SCHENCK[3] obtained adenine, oxalic acid, acetic acid, an acid having an unknown formula, and another acid which they call *martamic acid*, besides guanidine and urea. *Martamic acid* has the formula $C_5H_8N_6O_5$ or

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[1] Neumann, l. c.; Kostytschew, Zeitschr. f. physiol. Chem., **39**; Bang, Hofmeister's Beiträge, **5**; Schmiedeberg and Herlant, Arch. f. exp. Path. u. Pharm., **44**.

[2] Alsberg, Arch. f. exp. Path. u. Pharm., **51**; Noll, Zeitschr. f. physiol. Chem., **25**; Mathews, *ibid.*, **23**; Gulewitsch, *ibid.*, **27**; see also for the other references foot-note 2, p. 152.

[3] Kutscher and Schenck, Zeitschr. f. physiol. Chem., **44**; Kutscher and Seemann, Ber. d. d. chem. Gesellsch., **36**, and Centralbl. f. Physiol., **17**.

$C_5H_{10}N_4O_3$ and gives a silver salt which is soluble in ammonia or nitric acid, and which crystallizes in tufts of leaves. The crystalline acid, which is soluble in ether, sublimes at 150° and does not give the murexide test or Weidel's test.

Guanylic Acid. This acid, which thus far has been obtained only from the pancreas, has, according to BANG, the composition $C_{44}H_{66}N_{20}P_4O_{34}$. It is readily soluble in warm water, but partially separates out on cooling. It is considered as an ester of a glycerophosphoric acid and decomposes on hydrolytic cleavage with acids, according to BANG, into 4 molecules of guanine, 3 molecules of pentose (*l*-xylose according to NEUBERG), 3 molecules of glycerine, and 4 molecules of phosphoric acid.

According to the more recent investigations of BANG and RAASCHOU[1] the guanylic acid, which BANG now designates as β-acid is formed, in the preparation from another acid called α-guanylic acid, by the action of the alkali. The α-guanylic acid, which is readily soluble in water, even in cold water, contains less phosphorus and nitrogen (6.65 and 15.38 per cent respectively) as compared with the β-acid, which contains 7.64 per cent phosphorus and 18.21 per cent nitrogen. By the action of alkalies the α-guanylic acid splits off a pentose group and is converted into the β-acid.

The following acid is also generally included among the nucleic acids:

Inosinic acid, $C_{10}H_{13}N_4PO_8$, was first isolated by LIEBIG from the flesh of certain animals and then closely studied by HAISER.[2] It contains phosphorus, is amorphous, and gives crystalline salts with barium and calcium. HAISER obtained hypoxanthine as a cleavage product and probably also trioxyvalerianic acid, though this has not been positively proven.

The thymusnucleic acid may be prepared as the copper salt, according to SCHMIEDEBERG, from the heads of the salmon spermatozoa or from the residue after the peptic digestion of the thymus glands (HERLANT). The protamines are removed by the action of copper chloride and the last traces of proteid removed by dissolving the residue in dilute caustic potash and precipitating this solution with alcohol, and this is repeated until it fails to give the biuret test. The copper salt can be precipitated by copper chloride from the watery solution of the potassium nucleate, after acidification with acetic acid. According to NEUMANN, the two thymusnucleic acids, α and β, can be obtained from the gland, after previously boiling the same with water containing acetic acid and then cutting it up fine. The finely divided gland is boiled with about 3 per cent NaOH for one-half hour for the α-acid and two hours for the β-acid, and sodium acetate is added at the same time. After neutralization with acetic acid, filtration and concentration, the product is precipitated with alcohol. The nucleic acids can be obtained from the precipitated sodium nucleates by precipitating with alcohol containing hydrochloric acid. In the separation of the two acids, KOSTYTSCHEW makes use of the different behavior of the barium salts on saturating their solution with barium acetate (see above). LEVENE'S[3] method consists, on the contrary, in treating the organs first with 5 per cent sodium hydrate or with 8 per cent ammonia in the cold, then nearly neutralizing with acetic acid, precipitating the proteids with picric acid, and treating the strongly acidified liquid (acetic acid) with alcohol. In the presence of sufficient acetate the nucleic acids are precipitated. More recently LEVENE has suggested that the nucleic acid be dissolved in strong acetic acid and then precipitated with copper chloride or hydrochloric acid.

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[1] Hofmeister's Beiträge, 4.

[2] Liebig, Annal. d. Chem. u. Pharm., 62; F. Haiser, Monatshefte f. Chem., 16.

[3] Schmiedeberg, Arch. f. exp. Path. u. Pharm., 43; Herlant, *ibid.*, 44; Neumann,

Guanylic acid may be best prepared, according to BANG and RAASCHOU, by the following method: After treating the pancreas with 1 per cent sodium-hydrate solution for twenty-four hours at the room temperature, it is dissolved by warming, then made faintly acid with acetic acid, filtered, made faintly alkaline with ammonia, strongly concentrated, and precipitated with alcohol while hot. The proteoses remain in solution, and the precipitated guanylic acid (α-acid) is purified by repeated solutions in water and precipitations by alcohol.

Plant Nucleic Acids. Those best known are the yeast nucleic acid and the triticonucleic acid, $C_{41}H_{61}N_{16}P_4O_{31}$, isolated by OSBORNE and HARRIS from the wheat embryo, and which according to these investigators is identical with the yeast nucleic acid. The plant nucleic acids are nearly related to the thymonucleic acids, but differ from them by the fact that in the thymonucleic acids the pyrimidine groups are represented by uracil, cytosine, and thymine, and in the triticonucleic acid by cytosine and uracil. This last acid, which is dextrorotatory, yields on hydrolysis with acid 1 molecule of guanine, 1 molecule each of adenine and cytosine (WHEELER and JOHNSON [1]), 2 molecules of uracil, and 3 molecules of pentose for every 4 atoms of phosphorus. LEVENE has been able to prepare from the tubercle bacilli nucleic acids whose nature has not been closely studied.

Plasminic acid is an acid which was prepared by ASCOLI and KOSSEL [2] by the action of alkali upon yeast. It contains iron and is soluble in very dilute hydrochloric acid (1 p. m.). It is still a question whether it is a mixture or a chemical individual.

In regard to the preparation of yeast and triticonucleic acid we must refer to the works of ALTMANN, KOSSEL, OSBORNE and HARRIS.[3]

Among the cleavage products of the nucleic acids the purine derivatives and the pyrimidine derivatives are of special interest.

Purine Bases (nuclein bases, alloxuric bases, xanthine bodies). With these names we designate a group of bodies consisting of *carbon, hydrogen, nitrogen,* and in most cases also of *oxygen,* which, by their composition, show a relationship not only among themselves, but also with uric acid. All these bodies, uric acid included, are considered as consisting of an alloxuric and a urea nucleus, and for this reason KOSSEL and KRÜGER have called them *alloxuric bases,* or the entire group, including uric acid, *alloxuric bodies.* According to E. FISCHER,[4] who has not only shown, in several ways, the close relationship of uric acid to this group, but has also pre-

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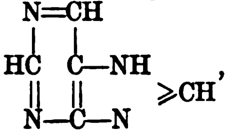
Arch. f. (Anat. u.) Physiol., 1899, Supplb.; Levene, Zeitschr. f. physiol. Chem., **32** and **45**; Kostytschew, *ibid.*, **39.**

[1] Amer. Chem. Journ., **29.**

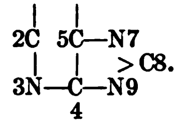
[2] Ascoli, Zeitschr. f. physiol. Chem., **28**

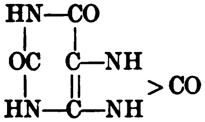
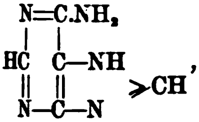
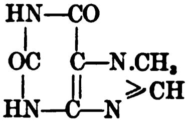
[3] See foot-note 2, p. 152.

[4] See Fischer, Ber. d. deutsch. chem. Gesellsch., **30** and **32.**

pared a number of the members of this group synthetically, they are all derived from a compound, $C_5H_4N_4$ =  , called *purine.*

The different purine bodies are derived therefrom by the substitution of the various hydrogen atoms by hydroxyl, amide, or alkyl groups. In order to signify the different positions of substitution **Fischer** has proposed to number the nine members of the purine nucleus in the following way:



For example, uric acid,  , is 2, 6, 8-trioxypurine, adenine,  , is 6-aminopurine, and heteroxanthine,  , is 7-methyl-2, 6-dioxypurine, etc.

The starting-point used by **Fischer** for the synthetical preparation of the purine bases was 2, 6, 8-trichlorpurine, which is obtained, with 8-oxy-2, 6-dichlorpurine as an intermediary product, from potassium urate and phosphorus oxychloride. The close relation between uric acid and the nuclein bases follows from the fact, as shown by **Sundvik**,[1] that two bodies may be obtained on the reduction of uric acid in alkaline solution, which, although not quite identical with xanthine and hypoxanthine, are at least very similar thereto. **Gautier** claims to have prepared xanthine synthetically by heating hydrocyanic acid with water and acetic acid. Further syntheses of purine bases have been made by **Traube**.[2]

The purine bodies or alloxuric bodies found in the animal body or its excreta are as follows: *Uric acid, xanthine, heteroxanthine, 1-methylxanthine, paraxanthine, guanine, epiguanine, hypoxanthine, episarkine, adenine,* and *carnine.* The bodies *theobromine, theophylline,* and *caffeine,* occurring in the vegetable kingdom, stand in close relationship to this group.

The composition of the purine bodies most important from a physiological standpoint is as follows:

Uric acid,	$C_5H_4N_4O_3$		2, 6, 8-trioxypurine
Xanthine,	$C_5H_4N_4O_2$		2, 6-dioxypurine
1-methylxanthine,	$C_6H_6N_4O_2$	1-methyl	" "
Heteroxanthine,	$C_6H_6N_4O_2$	7- "	" "
Theophylline,	$C_7H_8N_4O_2$	1, 3-dimethyl	" "
Paraxanthine,	$C_7H_8N_4O_2$	1, 7- "	" "
Theobromine,	$C_7H_8N_4O_2$	3, 7- "	" "
Caffeine,	$C_8H_{10}N_4O_2$	1, 3, 7-trimethyl	" "

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[1] Zeitschr. f. physiol. Chem., 23.

[2] Gautier, Compt. rend., 98, 1523, and Ber. d. deutsch. chem. Gesellsch., 31; W. Traube, *ibid.*, 33, and Annal. d. Chem. u. Pharm., 331.

Hypoxanthine,	$C_5H_4N_4O$	6-oxypurine
Guanine,	$C_5H_5N_5O$	2-amino " "
Epiguanine,	$C_6H_7N_5O$	7-methyl " " " "
Adenine,	$C_5H_5N_5$	6-aminopurine
Episarkine,	$C_4H_8N_4O_3$(?)	
Carnine,	$C_7H_8N_4O_3$	

After SALOMON [1] had shown the occurrence of xanthine bodies in young cells, the importance of the xanthine bodies as decomposition products of cell nuclei and of nucleins was shown by the pioneering researches of KOSSEL, who discovered adenine and theophylline. KOSSEL gave them the name nuclein bases. In those tissues in which, as in the glands, the cells have kept their original state, the nuclein bases are not found free, but in combination with other atomic groups (nucleins). In such tissues, on the contrary, as in muscles, which are poor in cell nuclei, the nuclein bases are found in the free state. Since the nuclein bases, as suggested by KOSSEL, stand in close relationship to the cell nucleus, it is easy to understand why the quantity of these bodies is so greatly increased when large quantities of nucleated cells appear in such places as were before relatively poorly endowed. As an example of this, the blood, in leucæmia, is extremely rich in leucocytes. In such blood KOSSEL [2] found 1.04 p. m. nuclein bases, against only traces in the normal blood. That the nuclein bases are also intermediate steps in the formation of urea or uric acid in the animal organism is probable, and will be shown later (see Chapter XV).

Only a few of the nuclein bases have been found in the urine or in the muscles. Only four bases—xanthine, guanine, hypoxanthine, and adenine—have been obtained, thus far, as cleavage products of nucleins. In regard to the purine bodies from other substances we refer the reader to their respective chapters. Only the above four bodies, the real nuclein bases, will be considered at this time.

Of these four bodies xanthine and guanine form one special group and hypoxanthine and adenine another. By the action of nitrous acid guanine is converted into xanthine and adenine into hypoxanthine.

$$\underset{\text{Guanine}}{C_5H_4N_4O.NH} + HNO_2 = \underset{\text{Xanthine}}{C_5H_4N_4O_2} + N_2 + H_2O;$$

$$\underset{\text{Adenine}}{C_5H_4N_4.NH} + HNO_2 = \underset{\text{Hypoxanthine}}{C_5H_4N_4O} + N_2 + H_2O.$$

Similar transformations may be brought about by putrefaction as well as by the action of special enzymes. The researches of SCHITTENHELM, LEVENE, JONES, PARTRIDGE, WINTERNITZ, and BURIAN [3] have shown that in various organs desamination enzymes, such as *guanase* and *adenase*, occur, which convert guanine and adenine into xanthine and hypoxanthine respectively, and also oxidases which oxidize hypoxanthine into xanthine and this then into uric acid.

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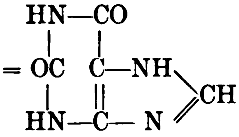
[1] Sitzungsber. d. Bot. Verein der Provinz Brandenburg, 1880.

[2] Zeitschr. f. physiol. Chem., 7.

[3] See Chapter XV (uric acid formation).

On cleavage with hydrochloric acid all four of the bodies are converted into ammonia, glycocoll, carbon dioxide, and formic acid. On oxidation with hydrochloric acid and potassium chlorate, xanthine, bromadenine, and bromhypoxanthine yield alloxan and urea; guanine yields guanidine, parabanic acid (an oxidation product of alloxan), and carbon dioxide. According to BURIAN[1] the nuclein bases give beautiful red products with diazo-compounds as long as the imide hydrogen in the 7th position (see structural formula above) is not substituted. As the nucleic acids do not react with the diazo compounds, BURIAN concludes that probably the nucleic-acid residue is combined with the imide hydrogen at position 7.

The nuclein bases form crystalline salts with mineral acids, which, with the exception of the adenine salts, are decomposed by water. They are easily dissolved by alkalies, while with ammonia their action is somewhat different. They are all precipitated from acid solution by phosphotungstic acid; they also separate as silver compounds on the addition of ammonia and ammoniacal silver-nitrate solution. These precipitates are soluble in boiling nitric acid of 1.1 specific gravity. All xanthine bodies are also precipitated by FEHLING's solution (see Chapter XV) in the presence of a reducing substance such as hydroxylamine (DRECHSEL and BALKE). Copper sulphate and sodium bisulphite may also be used to advantage in their precipitation (KRÜGER).[2] This behavior of the xanthine bases serves just as well as the behavior with the silver solution for their precipitation and preparation.

Xanthine, $C_5H_4N_4O_2 =$ 

$$\begin{array}{lll} \mathrm{HN{-}CO} & & \\ | \quad\ | & & \\ \mathrm{OC} \quad \mathrm{C{-}NH} & \searrow & \\ | \quad\ \| & & \mathrm{CH} \\ \mathrm{HN{-}C{-}\ N} & \nearrow & \end{array}$$

(2, 6-dioxypurine), is found in the muscles, liver, spleen, pancreas, kidneys, testicles, carp-sperm, thymus, and brain. It occurs in small quantities as a physiological constituent of urine, and it occasionally has been found as a urinary sediment, or calculus. It was first observed in such a stone by MARCET. Xanthine is found in larger amounts in a few varieties of guano (Jarvis guano).

Xanthine is amorphous, or forms granular masses of crystals, or may also, according to HORBACZEWSKI,[3] separate as masses of shining, thin, large rhombic plates with 1 mol water of crystallization. It is very slightly soluble in water, in 14 151–14 600 parts at 16° C., and in 1300–1500 parts at 100° C. (ALMÉN[4]). It is insoluble in alcohol or ether, but is readily dissolved by alkalies and with difficulty by dilute acids. With hydrochloric acid it gives a crystalline, difficultly soluble combination. With very little caustic soda it gives a readily crystallizable compound, which is easily dissolved by an excess of alkali. Xanthine dissolved in ammonia gives with silver nitrate an insoluble, gelatinous precipitate of silver xanthine. This precipitate is dissolved by hot nitric acid, and by this means an easily soluble crystalline double compound is formed. Xanthine in aqueous solution is precipitated on boiling with copper acetate. At ordinary temperatures xanthine is precipitated by mercuric chloride and by ammoniacal basic lead acetate. It is not precipitated by basic lead acetate alone.

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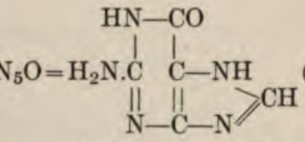
[1] Ber. d. d. chem. Gesellsch., 37.

[2] Balke, Zur Kenntnis der Xanthinkörper, Inaug.-Diss. Leipzig, 1893; Krüger, Zeitschr. f. physiol. Chem., 18.

[3] Zeitschr. f. physiol. Chem., 23.

[4] Journ. f. prakt. Chem., 96.

When evaporated to dryness in a porcelain dish with nitric acid, xanthine gives a yellow residue, which turns, on the addition of caustic soda, first red, and, after heating, purple-red. If we place some chloride of lime with some caustic soda in a porcelain dish and add the xanthine to this mixture, at first a dark-green and then quickly a brownish halo forms around the xanthine grains and finally disappears (Hoppe-Seyler). If xanthine is warmed in a small vessel on the water-bath with chlorine-water and a trace of nitric acid, and evaporated to dryness, and the residue is then exposed under a bell-jar to the vapors of ammonia, a red or purple-violet color is produced (Weidel's reaction). E. Fischer[1] has modified Weidel's reaction in the following way: He boils the xanthine in a test-tube with chlorine-water or with hydrochloric acid and a little potassium chlorate, then evaporates the liquid carefully and moistens the dry residue with ammonia.

Guanine, $C_5H_5N_5O =$ 

$$\begin{array}{l} \quad\quad\ \ HN\!-\!CO \\ \quad\quad\quad | \quad\ \ | \\ H_2N.C \quad C\!-\!NH \\ \quad\quad\ \| \quad\ \| \quad\quad > CH \\ \quad\quad\ N\!-\!C\!-\!N \end{array}$$

(2-amino-6-oxypurine). Guanine is found in organs rich in cells, such as the liver, spleen, pancreas, testicles, and in salmon-sperm. It is further found in the muscles (in very small amounts), in the scales and in the air-bladder of certain fishes as iridescent crystals of guanine-lime; in the retinal epithelium of fishes, in guano, and in the excrement of spiders it is found as chief constituent. It also occurs in human and pig urine. Under pathological conditions it has been found in leucæmic blood, and in the muscles, ligaments, and articulations of pigs with guanine-gout.

Guanine is a colorless, ordinarily amorphous powder which may be obtained as small crystals by allowing its solution in concentrated ammonia to spontaneously evaporate. According to Horbaczewski it may under certain conditions appear in crystals similar to creatinine zinc chloride. It is insoluble in water, alcohol, and ether. It is rather easily dissolved by mineral acids and readily by alkalies, but it dissolves with great difficulty in ammonia. According to Wulff[1] 100 c.c. of cold ammonia solution containing 1, 3, or 5 per cent NH_3 dissolve 9, 15, or 19 milligrams of guanine respectively. The solubility is relatively increased in hot ammonia solution. The hydrochloride readily crystallizes, and has been recommended by Kossel[2] for the microscopical detection of guanine, on account of its behavior to polarized light. The sulphate contains 2 molecules of water of crystallization, which is completely expelled on heating to 120° C., and this fact, as well as the fact that guanine yields guanidine on decomposition with chlorine-water, differentiates it from 6-amino-2-oxypurine, which is considered as an oxidation product of adenine and possibly occurs as a chemical metabolic product (E. Fischer). The 6-amino-2-oxypurine sulphate contains only 1 molecule of water of crystallization, which is not expelled at 120° C. Very dilute guanine solutions are precipitated by both picric acid and metaphosphoric acid. These precipitates may be used in the quantitative estimation of guanine. The silver compound dissolves with difficulty in boiling nitric acid, and on cooling the double compound crystallizes out readily. Guanine acts like xanthine in the nitric-acid test, but gives with alkalies on heating a more bluish-violet color. A warm solution of guanine hydrochloride gives with a cold saturated solution of picric acid a yellow precipitate consisting of silky needles (Capranica). With a concentrated solution of potassium bichromate a guanine solution gives a crystalline, orange-red precipitate, and with a concentrated solution of potassium ferricyanide a yellowish-brown, crystalline precipitate (Capranica). The composition of these and other guanine compounds has been studied by Kossel and Wulff.[3] Guanine does not give Weidel's reaction.

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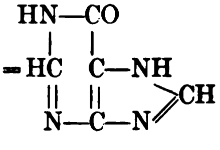
[1] Ber. d. deutsch. chem. Gesellsch., **30**, 2236.

Hypoxanthine, Sarkine, $C_5H_4N_4O$ =  = (6-oxypurine).

This body is found in the same tissues as xanthine. It is especially abundant in the sperm of the salmon and carp. Hypoxanthine occurs also in the marrow and in very small quantities in normal urine, and, as it seems, also in milk. It is found in rather considerable quantities in the blood and urine in leucæmia.

Hypoxanthine forms very small, colorless, crystalline needles. It dissolves with difficulty in cold water, but the statements in regard to the solubility therein are very contradictory.[1] It dissolves more readily in boiling water, in about 70–80 parts. It is nearly insoluble in alcohol, but is dissolved by acids and alkalies. The compound with hydrochloric acid is crystalline, and is more soluble than the corresponding xanthine derivative. It is easily soluble in dilute alkalies and ammonia. The silver compound dissolves with difficulty in boiling nitric acid. On cooling, a mixture of two hypoxanthine silver-nitrate compounds possessing an inconstant composition separates out. On treating this mixture with ammonia and an excess of silver-nitrate and heating, a silver hypoxanthine is formed, which when dried at 120° C. has a constant composition, $2(C_5H_2Ag_2N_4O)H_2O$, and is used in the quantitative estimation of hypoxanthine. Hypoxanthine picrate is soluble with difficulty, but if a boiling-hot solution of the same is treated with a neutral or only faintly acid solution of silver nitrate the hypoxanthine is nearly quantitatively precipitated as the compound $C_5H_3AgN_4O.C_6H_2(NO_2)_3OH$. Hypoxanthine does not yield an insoluble compound with metaphosphoric acid. When treated, like xanthine, with nitric acid it yields a nearly colorless residue which, on warming with alkali, does not turn red. Hypoxanthine does not give Weidel's reaction. After the action of hydrochloric acid and zinc a hypoxanthine solution becomes first ruby-red and then brownish red in color on the addition of an excess of alkali (Kossel). According to E. Fischer[2] a red coloration occurs even in the acid solution.

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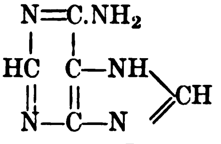
[1] Zeitschr. f. physiol. Chem., 17.

[2] Ueber die chem. Zusammensetz. der Zelle, Verh. d. physiol. Gesellsch. zu Berlin, 1890–91, Nos. 5 and 6.

[3] Zeitschr. f. physiol. Chem., 17; Capranica, *ibid.*, 4.

Adenine, $C_5H_5N_5=$  (6-aminopurine), **was first found** by Kossel[3] in the pancreas. It occurs in all nucleated cells, but in greatest quantities in the sperm of the carp and in the thymus. Adenine has also been found in leucæmic urine (Stadthagen[4]). It may be obtained in large quantities from tea-leaves.

Adenine crystallizes with 3 molecules of water of crystallization in long needles which become opaque gradually in the air, but much more rapidly when warmed. If the crystals are warmed slowly with a quantity of water insufficient for solution, they become suddenly cloudy at 53° C., a characteristic reaction for adenine. It dissolves in 1086 parts cold water, but is easily soluble in warm. It is insoluble in ether, but somewhat soluble in hot alcohol and easily so in acids and alkalies. It is more easily soluble in ammonia solution than guanine, but less soluble than hypoxanthine. The silver compound of adenine is difficultly soluble in warm nitric acid, and deposits on cooling as a crystalline mixture of adenine silver-nitrates. With picric acid adenine forms a compound, $C_5H_5N_5.C_6H_2(NO_2)_3OH$, which is very insoluble and which separates more readily than the hypoxanthine picrate and which can be used in the quantitative estimation of adenine. We also have an adenine mercury-picrate. Metaphosphoric acid with adenine gives a precipitate which dissolves in an excess of the acid if the solution is not too dilute. Adenine hydrochloride gives with gold chloride a double compound which consists in part of leaf-shaped aggregations and in part of cubical or prismatic crystals, often with rounded corners. This compound is used in the microscopic detection of adenine. With the nitric-acid test and with WEIDEL'S reaction adenine acts in the same way as hypoxanthine. The same is true for its behavior with hydrochloric acid and zinc with subsequent addition of alkali.

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[1] See E. Fischer, Ber. d. deutsch. chem. Gesellsch., 30.

[2] Kossel, Zeitschr. f. physiol. Chem., 12, 252; E. Fischer, l. c.

[3] See Zeitschr. f. physiol. Chem., 10 and 12.

[4] Virchow's Arch., 109.

The procedure for the preparation and detection of the four above-described xanthine bodies in organs and tissues is, according to KOSSEL and his pupils, as follows: The finely divided organ or tissue is boiled for three or four hours with sulphuric acid of about 5 p. m. The filtered liquid is freed from proteid by basic lead acetate, and the new filtrate is treated with sulphuretted hydrogen to remove the lead, again filtered, concentrated, and, after adding an excess of ammonia, precipitated with ammoniacal silver nitrate. The silver compound (with the addition of some urea to prevent nitrification) is dissolved in not too large a quantity of boiling nitric acid of sp. gr. 1.1, and this solution filtered boiling hot. On cooling, the silver xanthine remains in the solution, while the double compounds of guanine, hypoxanthine, and adenine crystallize out. The silver xanthine may be precipitated from the filtrate by the addition of ammonia and the xanthine set free by means of sulphuretted hydrogen. The three above-mentioned silver-nitrate compounds are decomposed in water with ammonium sulphide and heat; the silver sulphide is filtered off, the filtrate concentrated, saturated with ammonia, and digested on the water-bath. The guanine remains undissolved, while the other two bases pass into solution. A part of the guanine is still retained by the silver sulphide, and may be liberated by boiling it with dilute hydrochloric acid and then saturating the filtrate with ammonia. When the above filtrate containing the adenine and hypoxanthine, which has been, if necessary, freed from ammonia by evaporation, is allowed to cool, the adenine separates, while the hypoxanthine remains in solution. According to BALKE[1] we can advantageously precipitate the xanthine bases with a copper salt and hydroxylamine as above mentioned and then further separate the bodies. In cases where the proteids have not been completely separated it is advantageous to precipitate the bases as copper compounds with copper sulphate and bisulphite. KRÜGER and SCHITTENHELM'S[2] method for the separation and quantitative estimation of purine bodies in fæces can be followed and the bases then transformed into silver compounds.

The method of BURIAN and HALL[3] is serviceable in the estimation of the total quantity of purine bodies in animal organs; the quantitative estimation of the various bases is performed in the main according to the method above described. The xanthine is weighed as silver xanthine. The three silver-nitrate compounds are converted into the corresponding silver derivatives by ammonia and the addition of silver nitrate, and then ammonium sulphide is allowed to act upon the carefully washed silver compounds. The guanine is weighed as such. The ammoniacal filtrate containing the adenine and hypoxanthine, which must not be mixed with the hydrochloric-acid extract of the silver sulphide, is neutralized and a cold concentrated solution of sodium picrate added until the entire liquid has a pronouncedly yellow color. The adenine picrate is immediately filtered off, washed on the filter-paper with water, dried at above 100° C., and weighed. The filtrate containing the hypoxanthine is gradually treated while boiling hot with silver nitrate, and after cooling more silver nitrate is added to see if the precipitation is complete. The silver-hypoxanthine picrate is washed, dried at 100° C., and weighed. In regard to the composition of these compounds see pages 162 and 163. This method of separating adenine and hypoxanthine presupposes the absence of hydrochloric acid in the liquid.

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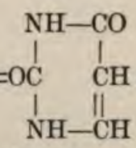
[1] Zeitschr. f. physiol. Chem., 45.

[2] Ibid., 38.

The above method of separation with ammonia does not give exact results on account of the not inconsiderable solubility of guanine in warm ammonia. According to Kossel and Wulff,[1] the guanine may therefore be precipitated from sufficiently dilute solutions by an excess of metaphosphoric acid and the nitrogen determined in the washed precipitate by Kjeldahl's method. The adenine and hypoxanthine may be precipitated from the filtrate by ammoniacal silver nitrate. The silver compound is decomposed with very dilute hydrochloric acid and the adenine separated from the hypoxanthine according to the suggestion of Bruhns.[2] In regard to the complications in the detection and exact estimation of purine bodies in extracts of organs, we refer to the works of His and Hagen and of Burian and Hall.[3]

Uracil, $C_4H_4N_2O_2=$ 

$$\begin{array}{lcl} NH & - & CO \\ | & & | \\ OC & & CH \\ | & & \| \\ NH & - & CH \end{array}$$

(2,6-dioxypyrimidine), was first obtained by Ascoli and Kossel from yeast nucleic acid and later prepared by Kossel and Steudel from thymusnucleic acid and herring testicles, by Levene from the spleen and pancreas nucleic acids, and by Levene and Mandel from the nucleic acid of the haddock roe. The synthetical preparation was first performed by E. Fischer and Roeder.[4]

Uracil crystallizes in needles which cluster in rosettes. On careful heating it sublimes in part undecomposed, but develops red vapors and decomposes in part. It is readily soluble in hot water but less so in cold water, and is nearly insoluble in alcohol and ether. It is readily soluble in ammonia. It is precipitated by silver-nitrate solution only after the careful addition of ammonia or baryta-water, as the precipitate is readily soluble in an excess of ammonia. Uracil responds to Weidel's test (p. 160). In regard to the preparation of uracil see Kossel and Steudel.[1]

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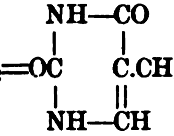
[1] Zeitschr. f. physiol. Chem., **17**.

[2] *Ibid.*, **14**, 559.

[3] His and Hagen, *ibid.*, **30**, and Burian and Hall, *ibid.*, **38**.

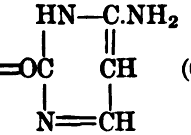
[4] Ascoli, *ibid.*, **31**; Kossel and Steudel, *ibid.*, **37**; Levene, *ibid.*, **38**, **39**; Levene and Mandel, *ibid.*, **49**; E. Fischer and Roeder, Ber. d. d. chem. Gesellsch., **34**.



Thymine, $C_5H_6N_2O_2$=OC C.CH_3 (5-methyluracil). This body, which is identical with *nucleosin* obtained by Schmiedeberg from salmonucleic acid, is obtained from the thymusnucleic acids and was first prepared by Kossel and Neumann from thymusnucleic acid, and then by other investigators, especially Levene and Mandel, from other animal nucleic acids. Fischer and Roeder and recently Gerngross[2] have prepared it synthetically.

Thymine crystallizes in small leaves grouped in stellar or dendriform clusters or, rarely, in short needles (Gulewitsch[3]). On heating it sublimes. It is difficultly soluble in cold water, more soluble in hot water, and insoluble in alcohol. It behaves like uracil towards ammonia or baryta-water and silver nitrate. Thymine is precipitated by phosphotungstic acid, which does not precipitate uracil. Bromine-water is decolorized by thymine, producing bromthymine. For its detection we make use of the sublimation, the behavior towards silver nitrate, and its elementary analysis.

In regard to the methods of preparation see Kossel and Neumann and W. Jones.[4]



Cytosine, $C_4H_5N_3O$=OC CH (6-amino-2-oxypyrimidine), was first prepared by Kossel and Neumann from thymusnucleic acid, and then by Kossel and Steudel, also by Levene and Mandel, from the spleen and many other animal nucleic acids, by Inouye and Kotake[5] from the nucleic acid of the intestine, and finally also by Wheeler and Johnson from triticonucleic acid. Wheeler and Johnson[6] have also prepared it synthetically.

The free base is difficultly soluble in water and crystallizes in thin leaves with a mother-of-pearl luster. The double compound with platinum chloride, the crystalline picrate, the nitrate, and the two sulphates are of importance in the detection of cytosine. This base is precipitated by phosphotungstic acid and by silver nitrate in the presence of an excess of barium hydroxide, which fact is of importance in the detection of cytosine (KUTSCHER). Cytosine gives, like uracil, the murexid reaction with chlorine-water and ammonia. In regard to the preparation of this base, see KOSSEL and STEUDEL and KUTSCHER.[1]

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[1] Zeitschr. f. physiol. Chem., **37**, 245.

[2] Schmiedeberg, l. c.; Arch. f. exp. Path. u. Pharm., **37**; Kossel and Neumann, Ber. d. d. chem. Gesellsch., **26** and **27**; Mandel and Levene, Zeitschr. f. physiol. Chem., **46**, **47**, **49**, **50**; E. Fischer and Roeder, *ibid.*, **34**; Gerngross, *ibid.*, **38**.

[3] Zeitschr. f. physiol. Chem., **27**.

[4] Kossel and Neumann, l. c., and W. Jones, Zeitschr. f. physiol. Chem., **29**, 461.

[5] In regard to the cited works see foot-note 2, p. 152.

[6] Amer. Chem. Journ., **29**; see also foot-note 2, p. 152.

The purine bases and the pyrimidine bodies are closely related to each other not only from a chemical but also from a physiological point of view, and for this reason the question has been repeatedly asked whether or not the pyrimidine bodies might not in part be products produced from the purine bodies by the action of acid. All researches thus far carried on to elucidate this question contradict such a possibility.

Mineral Bodies. The mineral substances found habitually in the cells of higher plants and of animals are potassium, sodium, calcium, magnesium, iron, phosphoric acid, chlorine, and perhaps also iodine (JUSTUS). In certain cells we also find manganese, silicic acid, arsenic, barium, and lithium.[2] We are chiefly indebted to LIEBIG for showing that the mineral bodies are as important for the normal constitution of the organs and tissues, as well as for the normal performance of the processes of life, as the organic constituents of the body. The importance of the mineral constituents is evident from the fact that we know no animal tissue and no animal fluid which is free from mineral bodies, and also from the fact that certain tissues or tissue elements contain chiefly certain mineral bodies and not others. In regard to the alkali compounds this division is, in general, as follows: The sodium compounds occur chiefly in the fluids, while the potassium compounds occur especially in the form-elements. Corresponding to this, the cells contain chiefly potassium as phosphate, while they are less rich in sodium and chlorine compounds. Still we have some exceptions to this rule, and it must be remarked that BEEBE[3] has found considerably more sodium than potassium in malignant tumors.

The importance of potassium for the life and the development of the cell has been shown by several observations. A very instructive and interesting example of this action has been shown by LOEB[4] in his investigations on the pathogenesis of the egg of the sea-annelide Chætopterus. The unfertilized eggs could, in sea-water alone, develop only to the eighth or sixteenth cell stage; after a short stay in sea-water to which KCl was added they developed to the trichophora larva. The fact that the KCl could not be replaced by other chlorides, but could be replaced by other potassium salts also shows that we are here dealing with a specific action of the potassium ions.

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[1] Kossel and Steudel, Zeitschr. f. physiol. Chem., **37** and **38**; Kutscher, *ibid.*, **38**.

[2] Justus, Virchow's Arch., **170** and **176**. In regard to arsenic see the works of Gautier, Compt. rend., **129**, **130**, **131**, **139**; Bertrand, *ibid.*, **134**; Segale, Zeitschr. f. physiol. Chem., **42**; Kunkel, *ibid.*, **44**. In regard to the barium see Schulze and Thierfelder, Sitzungsber. d. Gesellsch. naturforsch. Freunde, 1905, No. 1, and in regard to lithium see Hermann, Pflüger's Arch., **109**.

[3] Amer. Journ. of Physiol., **11** and **12**.

[4] *Ibid.*, **3**, **4**, and Pflüger's Arch., **87**.

The division of the potassium in cells and various tissues seems, according to MACALLUM,[1] to be peculiar and essentially different. According to MACALLUM the potassium is absent in the cell nuclei and in the head of spermatozoa as well as in nerve-cells and their axis-cylinders, while it occurs, on the contrary, in the medullary sheath and especially in the region of the nodes of RANVIER. A peculiar division of the potassium also occurs in the muscle fibers and secreting glandular cells.

The importance of phosphoric acid is not clear; it is possible that this acid is important for the formation of the lecithins and nucleins, and thereby indirectly makes possible the processes of growth and division, which are dependent upon the cell nucleus. LOEW[2] has shown, by means of cultivation experiments on algæ Spirogyra, that only by supplying phosphate (in his experiments potassium phosphate) was the nutrition of the cell nucleus made possible, and thereby the growth and division of the cells. The cells of the Spirogyra can be kept alive and indeed produce starch and proteins for some time without a supply of phosphates, but their growth and propagation suffer.

As both phosphoric acid and iron are obtained from the nuclein substances it is likely that these mineral bodies are, at least relatively, richest in the nucleus. As to the division of the mineral bodies between the protoplasm and the nucleus we know nothing with positiveness, and the same is true as to the form of combination of the mineral bodies in the nucleus. On incineration we obtain not only a mixture of the mineral bodies of the nucleus and protoplasm, but, as is true for all animal fluids and tissues, the original relationship is markedly changed. The combinations between the colloidal and mineral substances are destroyed, carbon dioxide discharged, and sulphuric acid and phosphoric acid may be produced from the organic bodies. The ordinary chemical analysis is not sufficient for the study of the mineral constituents of the fluids or tissue, their forms of combination and action; hence we must resort to physical-chemical methods.

According to the investigations carried on by these methods the conclusion has been reached, irrespective of the importance of the mineral bodies for the osmotic tension in the cells and tissues, that the part taken by the mineral bodies in cell life is essentially dependent upon the action of the ions. For example, the permeability of the blood-corpuscles as well as other cells for neutral alkali salts, which will be treated in the following chapter, shows an exchange of ions. The investigations of MAILLARD on the toxic action of copper salts and of PAUL and KÖRNIG[1] on that of mercury salts, acids, and alkalies offer other examples. From these investigations it follows that the toxicity is dependent upon the dissociation and that it is not dependent upon the total amount of, for example, copper or mercury salts present in the solution, but rather upon the number of copper or mercury ions.

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[1] Journ. of Physiol., 32.

[2] Biolog. Centralbl., 11, 269.

Beautiful and instructive examples of the importance of the ions for cell life have been shown by LOEB[2] and his collaborators. It is not within the scope of this book to give a detailed account of this important work, but perhaps it will be sufficient to give at least one example. The development of the eggs of the Fundulus can be retarded for a long time by a $\frac{5}{8}$ normal NaCl solution. On the addition of $CaSO_4$ this retardation is prevented and the development proceeds. Other calcium salts act like the sulphate, but alkali sulphates like Na_2SO_4 or other neutral alkali salts do not have this action, hence it must be a calcium ion action. Small quantities of other divalent cations, also trivalent ions, act in a similar way to calcium, while the salts of monovalent cations do not have this action. The fact that the fresh fertilized Fundulus eggs develop in distilled water as well as in sea-water shows that we are not dealing simply with a taking up of the salts necessary for development, but rather with an antagonistic salt action. They quickly die in a pure NaCl solution (having a concentration equal to that of sea-water); but if to the NaCl solution a small amount of zinc sulphate is added, the eggs are in condition to form an embryo. The common salt can also retard the toxic action of the zinc salt. According to LOEB every solution which contains only one electrolyte is poisonous, and this toxicity can be prevented by another electrolyte, and in certain cases by two other electrolytes. We are still undecided how the salts act in this regard; LOEB[3] believes that the antagonistic action of two salts may possibly be brought about by the fact that the diffusion in the egg is slower when the two are simultaneously in the solution than when each is alone in the solution. It is a difficult question to decide how the valence of the ions influences the power of certain ions to act as poisons or as anti-poisons.

The chief mass of the cells consists of colloids, and as the normal functions of the cells are connected with a certain physical condition of the protoplasm it is natural to consider the action of the ions in relationship to the change in the state of the colloids. The colloids can be precipitated by electrolytes, and the investigations of HARDY and PAULI[1] show that we are here probably also dealing with an ion action. Negatively charged colloids are, according to HARDY, precipitated by cations and positively charged by anions. A physiologically balanced salt mixture suitable for the normal functions may also be produced by the antagonism of the ion action in a complex solution containing several salts (LOEB and GIES). Changes in one or the other direction must correspondingly also bring about changes in the state of the colloid by the action of the ions. The action of ions in these cases, as well as the nature of colloids and the reasons for the change in their conditions, is a very difficult question, and its solution is still not answered.[2]

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[1] Maillard, Journ. de Physiol. et Path., **1**; Paul and Krönig, Zeitschr. f. physikal. Chem., **12**, and Zeitschr. f. Hygiene, **25**.

[2] Loeb, Amer. Journ. Physiol., **3**, **4**, and **6**; Pflüger's Arch., **80**, **87**, **88**, and **93** (with Gies) **97**, **101**, and **107**, and University of California Publications, Physiol., **1** and **2**. See also W. Oswald, Pflüger's Arch., **106**.

[3] Pflüger's Arch., **107**.

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[1] See foot-note 1 and 2, p. 168, and Mathews, Amer. Journ. of Physiol., 10 and 12.

[2] Hardy, Journ. of Physiol., 24, and Zeitschr. f. physikal. Chem., 33. See in regard to colloids Höber, Physikal. Chemie der Zelle und der Gewebe, Leipzig, 1906. Hamburger, Osmotischer Druck und Ionenlehre in den mediz. Wissenschaften, Bd. 3, 1904 and H. Aron, Biochem. Centralbl., 3, 505, and 4, 557.

CHAPTER VI.

THE BLOOD.

THE blood is to be considered from a certain standpoint as a fluid tissue, and it consists of a transparent liquid, the *blood-plasma*, in which a vast number of solid particles, the *red* and *white blood-corpuscles* (and the *blood-plates*), are suspended. We also find in the blood granules of different kinds, which are to be considered as transformation products of the form-elements.[1]

Outside of the organism the blood, as is well known, coagulates more or less quickly; but this coagulation is accomplished generally in a few minutes after leaving the body. All varieties of blood do not coagulate with the same degree of rapidity. Some coagulate more quickly, others more slowly. In vertebrates with nucleated blood-corpuscles (birds, reptiles, batrachia, and fishes) DELEZENNE has shown that the blood coagulates very slowly if it is collected under precautions so that it does not come in contact with the tissues. On contact with the tissues or with tissue extracts it coagulates in a few minutes. The blood with non-nucleated blood-corpuscles (mammals) coagulates, on the contrary, very rapidly. The coagulation of the blood in these cases may also be somewhat retarded by preventing the blood from coming in contact with the tissues (SPANGARO, ARTHUS[2]). Among the varieties of blood of mammals thus far investigated the blood of the horse coagulates most slowly. The coagulation may be more or less retarded by quickly cooling; and if we allow equine blood to flow directly from the vein into a glass cylinder which is not too wide and which has been cooled, and let it stand at 0° C., the blood may be kept fluid for several days. An upper amber-yellow layer of plasma gradually separates from a lower red layer composed of blood-corpuscles with only a little plasma. Between these is observed a whitish-gray layer which consists of white blood-corpuscles.

The plasma thus obtained and filtered is a clear amber-yellow alkaline

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[1] See Latschenberger, Wien. Sitzungsber., 105.

[2] Delezenne, Compt. rend. Soc. de biol., 49, Spangaro, Arch. ital. de Biol., 32; Arthus, Journ. de Physiol. et Pathol., 4.

(towards litmus) liquid which remains fluid for some time when kept at 0° C., but soon coagulates at the ordinary temperature.

The coagulation of the blood may be prevented in other ways. After the injection of peptone, or, more correctly, proteose solutions into the blood (in the living dog), the blood does not coagulate on leaving the veins (FANO, SCHMIDT-MÜLHEIM[1]). The plasma obtained from such blood by means of centrifugal force is called *peptone-plasma*. According to ARTHUS and HUBER[2] the caseoses and gelatoses act similarly to fibrin proteose in dogs. Eel serum and certain lymph-forming extracts of organs (see Chapter VII) also have an analogous action. The coagulation of the blood of warm-blooded animals is prevented by the injection of an effusion of the mouth of the officinal leech or a solution of the active substance of such an infusion, *herudin* (FRANZ), into the blood current (HAYCRAFT[3]). If the blood is allowed to flow directly, while stirring it, into a neutral salt solution—best a saturated magnesium-sulphate solution (1 vol. salt solution and 3 vols. blood)—we obtain a mixture of blood and salt which remains uncoagulated for several days. The blood-corpuscles, which, because of their adhesiveness and elasticity, would otherwise pass easily through the pores of the filter-paper, are made solid and stiff by the salt, so that they may be easily filtered. The plasma thus obtained, which does not coagulate spontaneously, is called *salt-plasma*.

An especially good method of preventing coagulation of blood consists in drawing the blood into a dilute solution of potassium oxalate, so that the mixture contains 0.1 per cent oxalate (ARTHUS and PAGÈS[4]). The soluble calcium salts of the blood are precipitated by the oxalate, and hence the blood loses its coagulability. On the other hand, HORNE[5] found that chlorides of calcium, barium, and strontium, when present in large amounts (2-3 per cent), may prevent coagulation for several days. According to ARTHUS[6] a non-coagulable blood-plasma may be obtained by drawing the blood into a sodium-fluoride solution until it contains 0.3 per cent NaFl.

On coagulation there separates in the previously fluid blood an insoluble or a very difficultly soluble protein substance, *fibrin*. When this separation takes place without stirring, the blood coagulates in a solid mass which, when carefully severed from the sides of the vessel, contracts, and a clear, generally yellow-colored liquid, the *blood-serum*, exudes. The solid coagulum which encloses the blood-corpuscles is called the *blood-clot* (placenta sanguinis). If the blood is beaten during coagulation, the fibrin separates in elastic threads or fibrous masses, and the *defibrinated blood* which separates is sometimes called *cruor*,[1] and consists of blood-corpuscles and blood-serum, while uncoagulated blood consists of blood-corpuscles and blood-plasma. The essential chemical difference between blood-serum and blood-plasma is that the blood-serum does not contain even traces of the mother-substance of fibrin, the fibrinogen, which exists in the blood-plasma, and the serum is proportionally richer in another body, the fibrin ferment (see page 175).

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[1] Fano, Arch. f (Anat. u) Physiol., 1881; Schmidt-Mülheim, *ibid.*, 1880.

[2] Arch. de Physiol. (5), 8.

[3] Haycraft, Proc. Physiol. Soc , 1884, 13, and Arch. f. exp. Path. u. Pharm., **18**; Franz, Arch. f. exp. Path. u. Pharm., **49**.

[4] Archives de Physiol. (5), **2**, and Compt. rend., **112**.

[5] Journ. of Physiol., **19**.

[6] Journ. de Physiol. et Pharm., **3** and **4**.

I. BLOOD-PLASMA AND BLOOD-SERUM.

The Blood-plasma.

In the coagulation of the blood a chemical transformation takes place in the plasma. A part of the proteins separates as insoluble fibrin. The albuminous bodies of the plasma must therefore be first described. They are, as far as we know at present, *fibrinogen, nucleoproteid, serglobulins*, and *seralbumins*.

Fibrinogen occurs in blood-plasma, chyle, lymph, certain transudates and exudates, in bone-marrow (P. MÜLLER), and perhaps also in other lymphoid organs. The seats of formation of fibrinogen are, according to MATHEWS, the leucocytes, especially of the intestine, according to MÜLLER, the bone-marrow and probably other lymphoid organs such as the spleen and lymph glands, and according to DOYON and NOLF, the liver. The statement that the intestinal wall is a seat of formation of fibrinogen, a view that had already been held by DASTRE, is substantiated not only by the direct researches of MATHEWS, but also by the older and confirmed statement that the blood from the mesentery vein is richer in fibrinogen than the arterial blood. The occurrence of fibrinogen in the bone-marrow, as shown by MÜLLER, and an increase of fibrinogen in the blood as well as in the bone-marrow of animals immunized with certain bacteria, especially pus-staphylococci, indicates the formation of fibrinogen in this tissue. That the liver takes part in the formation of fibrinogen is made probable by the fact that the quantity of fibrinogen in the blood strongly diminishes after the extirpation of the liver (NOLF), and that fibrinogen may indeed be entirely absent in the blood in phosphorus poisoning (CORIN and ANSIAUX, JACOBY, DOYON, MOREL, and KAREFF[2]).

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[1] The name *cruor* is used in different senses. We sometimes mean thereby only the blood when coagulated in a red solid mass, in other cases the blood-clot after the separation of the serum, and again the sediment consisting of red blood-corpuscles which is obtained from defibrinated blood by means of centrifugal force or by letting it stand.

[2] P. Müller, Hofmeister's Beiträge, **6**; Mathews, Amer. Journ. of Physiol., **3**; Nolf, Bull. Acad. Roy. Belg., 1905, and Arch. intern. de Physiol., **3**, 1905; Corin and Ansiaux,

Fibrinogen has the general properties of the globulins, but differs from other globulins as follows: In a moist condition it forms white flakes which are soluble in dilute common salt solutions, and which easily conglomerate into tough, elastic masses or lumps. The solution in 5–10 per cent NaCl coagulates on heating at 52–55° C., and the faintly alkaline or nearly neutral weak salt solution coagulates at 56° C., or at exactly the same temperature at which the blood-plasma coagulates. Fibrinogen solutions are precipitated by an equal volume of a saturated common salt solution, and are completely precipitated by adding an excess of NaCl in substance (thus differing from serglobulin). A salt-free solution of fibrinogen in as little alkali as possible gives with $CaCl_2$ a precipitate which contains calcium and soon becomes insoluble. In the presence of NaCl or by the addition of an excess of $CaCl_2$ the precipitate does not appear.[1] A neutral solution of fibrinogen is precipitated by a concentrated solution of sodium fluoride when added in sufficient quantity. Fibrinogens from different kinds of blood behave somewhat differently in this regard. According to Huiskamp[2] fibrinogen from horse-blood hardly dissolves in NaCl of 3–5 per cent at ordinary temperatures, while it does dissolve at 40–45°. It also dissolves in ammonia of 0.05 per cent, and on the addition of 3–5 per cent NaCl this solution can be neutralized. The fibrinogen prepared by Huiskamp in this way retained its typical properties. Fibrinogen differs from the myosin of the muscles, which coagulates at about the same temperature, and from other protein bodies, in the property of being converted into fibrin under certain conditions. Fibrinogen has a strong decomposing action on hydrogen peroxide. It is quickly made insoluble by precipitation with water or with dilute acids. Its specific rotation is $(\alpha)_D = -52.5°$ according to Mittelbach.[3]

Fibrinogen may be easily separated from the salt-plasma or oxalate-plasma by precipitation with an equal volume of a saturated NaCl solution. For further purification the precipitate is pressed, redissolved in an 8 per cent salt solution, the filtrate precipitated by a saturated salt solution as above, and after being treated in this way three times, the precipitate at last obtained is pressed between filter-paper and finely divided in water. The fibrinogen dissolves with the aid of the small amount of NaCl contained in itself, and the solution may be made salt-free by dialysis with very faintly alkaline water. The fibrinogen can be nearly freed from fibrin-globulin, which will be spoken of later, by precipitating with double the volume of saturated sodium-fluoride solution, redissolving in water

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Maly's Jahresber., 24; Jacoby, Zeitschr. f. physiol. Chem., 30; Doyon, Morel, and Kareff, Compt. rend., 140; Doyon, Morel, and Péju, Compt. rend. soc. biolog., 58.

[1] See Hammarsten, Zeitschr. f. physiol. Chem., 22; Cramer, *ibid.*, 23.

[2] Huiskamp, *ibid.*, 44 and 46. In regard to fibrinogen the reader is referred to the author's investigations. Pflüger's Archiv, 19 and 22, and Zeitschr. f. physiol. Chem., 28.

[3] Zeitschr. f. physiol. Chem., 19.

with 0.05 per cent ammonia, and then neutralizing this solution treated with NaCl, and repeating this several times. Fibrinogen may also, according to Reye,[1] be prepared by fractionally precipitating the plasma with a saturated solution of ammonium sulphate. We have no knowledge as to the purity of the fibrinogen so prepared. From transudates we ordinarily obtain a fibrinogen which is strongly contaminated with lecithin and which can hardly be purified without decomposing it. The methods for the detection and quantitative estimation of fibrinogen in a liquid were formerly based on its property of yielding fibrin on the addition of a little blood, of serum, or of fibrin ferment. Reye has suggested the fractional precipitation with ammonium sulphate as a quantitative method. The value of this method has not been sufficiently tested.

Fibrinogen stands in close relationship to its transformation product, fibrin.

Fibrin is the name of that protein body which separates on the so-called spontaneous coagulation of blood, lymph, and transudates as well as in the coagulation of a fibrinogen solution after the addition of serum or fibrin ferment (see below).

If the blood is beaten during coagulation, the fibrin separates in elastic, fibrous masses. The fibrin of the blood-clot may be beaten to small, less elastic, and not particularly fibrous lumps. The typical fibrous and elastic white fibrin, after washing, stands, in regard to its solubility, close to the coagulated proteins. It is insoluble in water, alcohol, or ether. It expands in hydrochloric acid of 1 p. m., as also in caustic potash or soda of 1 p. m., to a gelatinous mass, which dissolves at the ordinary temperature only after several days; but at the temperature of the body it dissolves more readily although still slowly. Fibrin may be dissolved by dilute salt solutions after a long time at the ordinary temperature or much more readily at 40° C., and this solution takes place, according to Arthus and Huber and also Dastre,[2] without the aid of micro-organisms. This action is due to proteolytic enzymes carried down by the fibrin or enclosed within the leucocytes (Rulot[3]). According to Green and Dastre[4] two globulins are formed in the solution of fibrin in neutral salt solution, and according to Rulot also proteoses (and peptones) on the solution of fibrin containing leucocytes. Fibrin, like fibrinogen, decomposes hydrogen peroxide, due to a contamination with catalases, but this property is destroyed by heating or by the action of alcohol.

What has been said of the solubility of fibrin relates only to the typical fibrin obtained from the arterial blood of mammals or man by whipping and washing first with water and with common salt solution and then with water again. The blood of various kinds of animals yields fibrin with somewhat different properties, and according to FERMI[1] pig-fibrin dissolves much more readily than ox-fibrin in hydrochloric acid of 5 p. m.. Fibrins of varying purity or originating from blood from different parts of the body have unlike solubilities.

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[1] W. Reye, Über Nachweis und Bestimmung des Fibrinogens, Inaug.-Diss. Strassburg, 1898.

[2] Arthus and Huber, Arch. de Physiol. (5), 5; Dastre, *ibid.* (5), 7.

[3] Arch. intern. de Physiol., 1.

[4] Green, Journ. of Physiol., 8; Dastre, l. c.

The fibrin obtained by beating the blood and purified as above described is always contaminated by secluded blood-corpuscles or remains thereof, and also by lymphoid cells. It can be obtained pure only from filtered plasma or filtered transudates. For the pure preparation, as well as for the quantitative estimation of fibrin, the spontaneously coagulating liquid is at once, or the non-spontaneously coagulating liquid only after the addition of blood-serum or fibrin ferment, thoroughly beaten with a whalebone, and the separated coagulum is washed first in water and then with a 5 per cent common salt solution, and again with water, and finally extracted with alcohol and ether. If the fibrin is allowed to stand for some time in contact with the blood from which it was formed, it partly dissolves (*fibrinolysis*—DASTRE[2]). This fibrinolysis must be prevented in the exact quantitative estimation of fibrin (DASTRE). The blood constituents that are active in fibrinolysis are still not known, but they are without doubt of enzymotic nature. It must be mentioned that a strong fibrinolysis takes place in blood after acute phosphorus-poisoning (JACOBY and others), after extirpation of the liver (NOLF), and also when the coagulability of the blood has been reduced by the injection of proteoses (NOLF, RULOT[3]).

A pure fibrinogen solution may be kept at the ordinary temperature until putrefaction begins without showing a trace of fibrin coagulation. But if to this solution is added a water-washed fibrin-clot or a little blood-serum, it immediately coagulates and may yield perfectly typical fibrin. The transformation of the fibrinogen into fibrin requires the presence of another body contained in the blood-clot and in the serum. This body, whose importance in the coagulation of fibrin was first observed by BUCHANAN,[4] was later rediscovered by ALEXANDER SCHMIDT[5] and designated as *fibrin ferment* or *thrombin*. The nature of this enzymotic body has not been ascertained with certainty. Although many investigators, especially English, consider fibrin ferment as a globulin, still more recent experiments of PEKELHARING and others show that it is a nucleoproteid which according to HUISKAMP[6] occurs in the thymus gland partly as nucleohistone and partly in another form. Fibrin ferment is produced, according to PEKELHARING, by the influence of soluble calcium salts on a preformed zymogen existing in the non-coagulated plasma. SCHMIDT admits of the presence of such a mother-substance of the fibrin ferment in the blood and calls it *prothrombin*. The conversion of this mother-substance into thrombin requires, according to more recent investigations, the presence of a second, zymoplastic-acting substance (see Coagulation of the Blood). Thrombin is like other enzymes in that the very smallest amount of it produces an action and its solution becomes inactive on heating. The velocity of coagulation is dependent upon the quantity of thrombin, and FULD has found that at least within certain limits an increase of double the quantity of enzyme causes an increase of the coagulation velocity to one and one half. This is true only for experiments with plasma and solutions containing kinases (see Coagulation of the Blood), and MARTIN[1] has found another law from experiments with plasma and snake-poisons containing thrombin. According to him the behavior is as follows: As in the casein coagulation with rennin, the velocity of coagulation is inversely proportional to the quantity of ferment. The optimum of the thrombin action lies at about 40° C.; at 70–75° C. the enzyme is destroyed. The question as to whether the thrombin found in different animals is the same substance or whether we have several thrombins has not been decided. The latter is not improbable; nevertheless a definite specificity of different thrombins has not been observed with certainty.

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[1] Zeitschr. f. Biologie, 28.

[2] Archives de Physiol. (5), 5 and 6.

[3] Jacoby, Zeitschr. f. physiol. Chem., 30; Nolf, Arch. intern. de Physiol., 3, 1905; Rulot, l. c.

[4] London Med. Gazette, 1845, 617. Cit. by Gamgee, Journal of Physiol., 1879.

[5] Pflüger's Arch., 6; see also Zur Blutlehre, 1892, and Weitere Beiträge zur Blutlehre, 1895.

[6] Pekelharing, Verhandl. d. kon. Akad. d. Wetensch. te Amsterdam, 1892, Deel 1;

The isolation of thrombin has been tried in several ways. Ordinarily it may be prepared by the following method, proposed by ALEX. SCHMIDT.[2] Precipitate the serum or defibrinated blood with 15–20 vols. of alcohol and allow it to stand a few months. The precipitate is then filtered and dried over sulphuric acid. The ferment may be extracted from the dried powder by means of water. Other methods have been suggested by HAMMARSTEN and by PEKELHARING.[3]

The preparation of a thrombin solution as free as possible from lime may be accomplished by removing the lime salts from the serum by means of oxalate and precipitating the serum with alcohol and allowing it to stand under alcohol for several months. The dried powder is rubbed with water and freed from soluble salts by repeated lixiviation with water and by the use of centrifugal force. Then each gram of powder is allowed to stand some time with 100–150 c.c. water, is filtered, and in this way a solu-

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ibid., 1895, and Centralbl. f. Physiol., **9**; Wright, Proc. Roy. Irish Acad. (3), **2**, The Lancet, 1892, and On Wooldridge's Method, etc., British Med. Journal, 1891; Lilienfeld, Hämatol. Untersuch., Arch. f. (Anat. u.) physiol., 1892; Über Leukocyten und Blutgerinnung, *ibid.*; Halliburton and Brodie, Journal of Physiol., **17** and **18**; Huiskamp, Zeitschr. f. physiol. Chem., **32**; Pekelharing and Huiskamp, *ibid.*, **39**.

[1] Martin, Journ. of Physiol., **32**; Fuld, Hofmeister's Beiträge, **2**.

[2] Pflüger's Arch., **6**.

[3] Hammarsten, *ibid.*, **18**; Pekelharing, l. c.

tion is obtained which contains only about 0.3–0.4 p. m. solids and about 0.0007 p. m. CaO (HAMMARSTEN).

If a fibrinogen solution containing salt, as above prepared, is treated with a solution of fibrin ferment, it coagulates at the ordinary temperature more or less quickly and yields a typical fibrin. Besides the fibrin ferment the presence of neutral salts is necessary, for ALEX. SCHMIDT has shown that fibrin coagulation does not take place without them. The presence of soluble calcium salts is not, as is generally assumed, a positive condition for the formation of fibrin, because, as shown by ALEX. SCHMIDT, PEKELHARING, and HAMMARSTEN,[1] thrombin can transform fibrinogen into typical fibrin in the absence of lime salts precipitable by oxalate. The fibrin is not richer in lime than the fibrinogen (HAMMARSTEN) used to prepare it if the fibrinogen and thrombin solutions are employed as lime-free as possible, and the view that the fibrin formation is connected with a taking up of lime has been shown to be untenable. The quantity of fibrin obtained on coagulation is always smaller than the amount of fibrinogen from which the fibrin is derived, and we always find a small amount of protein substance in the solution. It is therefore not improbable that the fibrin coagulation, in accordance with the views first proposed by DENIS, is a cleavage process in which the soluble fibrinogen is split into an insoluble protein, the fibrin, which forms the chief mass, and a soluble protein substance which is produced only in small amounts. We find a globulin-like substance which coagulates at about 64° C. in blood-serum as well as in the serum from coagulated fibrinogen solutions. This substance is called *fibrin-globulin* by HAMMARSTEN. The recent investigations of HUISKAMP have shown that this substance is not formed as a cleavage product from pure fibrinogen but occurs in plasma or in fibrinogen solutions not purified of sodium fluoride beside the fibrinogen, or perhaps in loose combination with fibrinogen. The view that a cleavage takes place in the coagulation of the fibrinogen has not been supported by these investigations.[2]

There exist also other views in regard to the processes of coagulation in the formation of fibrin which are even less positively founded. The fact that the soluble lime salts are not necessary for the transformation of fibrinogen into fibrin is not in contradiction to the other fact that they must be present in the coagulation of blood or plasma. This apparent contradiction may be explained, as shown later, by the special condition of the blood-plasma, and we must not overlook the fact that the coagulation of the blood is a much more complicated process than the coagulation of a fibrinogen solution, inasmuch as the first involves other important questions, as, for instance, the reason for the blood remaining fluid in the body, the origin of the fibrin ferment, the importance of the form-elements in the coagulation etc. A fuller discussion of the various hypotheses and theories concerning the coagulation of the blood must therefore be given later.

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[1] See Hammarsten, Zeitschr. f. physiol. Chem., 22, which also cites the works of Schmidt and Pekelharing, and *ibid.*, 28.

[2] See Hammarsten, Zeitschr. f. physiol. Chem., 28; Heubner, Arch. f. exp. Path. u. Pharm., 49, and Zeitschr. f. physiol. Chem., 45; Huiskamp, *ibid.*, 44 and 46.

Nucleoproteid. This substance, which, as above mentioned, is considered by PEKELHARING and HUISKAMP as identical with the prothrombin or thrombin, occurs in the blood-plasma as well as in the serum, and is precipitated from the latter with the globulin. It is similar to the globulin in that it is readily soluble in neutral salt solution and can be completely salted out on saturation with magnesium sulphate and separates only incompletely on dialysis. It is much less soluble than serglobulin in an excess of dilute acetic acid and coagulates at 65–69° C. The difficulty of solution in acetic acid is used by PEKELHARING as an important means of separating the compound proteids from the globulins.

Serglobulins, also called *paraglobulin* (KÜHNE), *fibrinoplastic substance* (ALEX. SCHMIDT), *serum-casein* (PANUM[1]), occur in the plasma, serum, lymph, transudates and exudates, in the white and red corpuscles, and probably in many animal tissues and form-elements, though in small quantities. They are also found in the urine in many diseases.

The so-called serglobulin is without doubt not an individual substance, but consists of a mixture of two or more protein bodies which cannot be completely and positively separated from each other. The mixture of globulins obtained from blood-plasma or blood-serum by saturation with magnesium sulphate or half-saturation with ammonium sulphate consists of nucleoproteid, fibrin-globulin, and the true serglobulin or mixture of globulins.

The nucleoproteid has already been discussed. The fibrin-globulin, which occurs in the serum only in small amounts, can be completely precipitated by NaCl. It has the general properties of the globulins, but differs from the serglobulins by a lower coagulation temperature, 64–66° C., and also in that it is precipitated by $(NH_4)_2SO_4$ even at 28 per cent saturation.

Serglobulins. If the globulin obtained by saturation with magnesium sulphate is dialyzed, then, as has been known for a long time and further substantiated by MARCUS, only a part of the globulin separates out, while a portion remains in solution and cannot be precipitated by the addition of acid. For this reason MARCUS[2] also differentiates between a water-soluble globulin and one insoluble in water. According to the recent investigations of HOFMEISTER and PICK[3] the part insoluble in water corresponds chiefly to a globulin fraction readily precipitated by $(NH_4)_2SO_4$ (by 28–36 vols.

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[1] Kühne, Lehrbuch d. physiol. Chem., Leipzig, 1866–68; Alex. Schmidt, Arch. f. (Anat. u.) Physiol., 1861–62; Panum, Virchow's Arch., **3** and **4**.

[2] Zeitschr. f. physiol. Chem., **28**.

[3] Hofmeister's Beiträge, **1**.

per cent saturated solution), and the part soluble in water corresponds to a more difficultly precipitable fraction (by 36–44 vols. per cent saturated solution). The first fraction is called *euglobulin* and the second *pseudoglobulin*. According to PORGES and SPIRO[1] the serglobulins can be separated by $(NH_4)_2SO_4$ into three fractions whose precipitation limits are 28–36, 33–42, and 40–46 vols. per cent saturated solution. All three fractions contain globulin insoluble in water. FREUND and JOACHIM[2] have recently found that the euglobulin as well as the pseudoglobulin fraction is a mixture of globulin soluble in water and globulin insoluble in water, and consequently the number of different globulins in the serum may be still greater.

It follows from all these investigations that either the difference between the globulin soluble in water and that insoluble is not sufficient or that the fractional precipitation with ammonium sulphate is not suited for the separation of the various globulins. This latter seems to be the case, as shown by HASLAM.[3] It must not be forgotten that the globulin fractions are always contaminated with other serum constituents and that these may influence the solubilities and precipitability. As HAMMARSTEN has shown, a water-soluble globulin can be transformed into a globulin insoluble in water by careful purification, and also the reverse, namely, a globulin insoluble in water can sometimes be converted into one soluble in water by allowing it to lie in the air. An insoluble protein like casein can also, according to HAMMARSTEN,[4] have the solubilities of a globulin due to contamination with constituents of the serum, and K. MÖRNER[5] has also shown that a contamination of the serum-globulins with soap can essentially modify the precipitation of these globulins. Under these circumstances the above statements in regard to the different globulin fractions must be accepted with great caution.

The investigations made thus far upon the so-called serglobulin have not led to any positive results. That this globulin, with the exception of the enzymes, immune bodies, and other unknown substances which are carried down by the various fractions, is a mixture of globulins there seems to be no doubt. The serglobulin or the globulin mixture which is obtained from the serum by the methods to be described has the following properties.

In a moist condition it forms snow-white flaky masses, neither tough nor elastic, which always contain thrombin and hence can bring about coagulation in a fibrinogen solution. The neutral solution is only incompletely precipitated by NaCl added to saturation and is not precipitated by an equal volume of a saturated salt solution. It is only partly precipitated by dialysis or by the addition of acid. On saturation with magnesium sulphate or one-half saturation with ammonium sulphate a complete precipitation is obtained. The coagulation temperature is, with 5–10 per cent NaCl in solution, 69–76°, but more often 75° C. The specific rotation of the solution containing salt is $(\alpha)_D = -47.8^\circ$ for the serglobulin from ox-blood (Fredericq[1]). The various globulin fractions do not differ essentially from each other in their coagulation temperatures, specific rotation, refraction coefficient (Reiss[2]), and their elementary composition. The average composition is, according to Hammarsten, C 52.71, H 7.01, N 15.85, S 1.11 per cent. K. Mörner[3] found 1.02 per cent sulphur and 0.67 per cent lead-blackening sulphur. All the sulphur seems to exist as cystine.

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[1] Hofmeister's Beiträge, 3.

[2] Zeitschr. f. physiol. Chem., 36.

[3] Journ. of Physiol., 32.

[4] See Hammarsten, Ergebnisse d. Physiol., 1, Abt. 1.

[5] Zeitschr. f. physiol. Chem., 34.

Serglobulin contains, as K. Mörner first showed, a carbohydrate group which can be split off. Langstein[4] has obtained several carbohydrates from the blood-globulin, namely, dextrose, glucosamine, and carbohydrate acids of unknown kinds. It has not been shown whether these small amounts of carbohydrate are derived from the globulin or from other contaminating bodies. According to Zanetti the blood-serum contains a glucoproteid, and the investigations of Eichholz[5] seem to show that the globulins are contaminated by a glucoproteid. According to Langstein the sugar is not only mixed with the globulin, but it exists in a combined form, probably in loose combination.

Serglobulin (the euglobulin) may be easily separated as a fine flocculent precipitate from blood-serum by neutralizing or making faintly acid with acetic acid and then diluting with 10–20 vols. of water. For further purification this precipitate is dissolved in dilute common salt solution, or in water by the aid of the smallest possible amount of alkali, and then reprecipitated by diluting with water or by the addition of a little acetic acid. All the serglobulin may also be separated from the serum by means of magnesium or ammonium sulphate; in these cases it is difficult to completely remove the salt by dialysis. As long as we are not agreed as to the number of globulins in the serum, it is not necessary to give a method of separating the various globulins in this mixture. Thus far the fractional precipitation with $(NH_4)_2SO_4$ has been used chiefly. The serglobulin from blood-serum is always contaminated by lecithin and thrombin. A serglobulin free from thrombin may be prepared from ferment-free transudates, as sometimes from hydrocele fluids, and this shows that serglobulin and thrombin are different bodies. For the detection and the quantitative estimation of serglobulin we may use the precipitation by magnesium sulphate added to saturation (Hammarsten), or by an equal volume of a saturated *neutral* ammonium-sulphate solution (Hofmeister and Kauder and Pohl[6]). In the quantitative estimation the precipitate is collected on a weighed filter, washed with the salt solution employed, dried with the filter at about 115° C., then washed with boiling-hot water, so as to completely remove the salt, extracted with alcohol and ether, dried, weighed and incinerated to determine the ash. The accuracy of these methods is questionable, as shown by the researches of HASLAM.

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[1] Bull. Acad. Roy. de Belg. (2), **50**. In regard to paraglobulin, see Hammarsten. Pflüger's Arch., **17** and **18**, and Ergebnisse d. Physiol., **1**, Abt. 1.

[2] Hofmeister's Beiträge, 4.

[3] Zeitschr. f. physiol. Chem., **34**.

[4] Mörner, Centralbl. f. Physiol., **7**; Langstein, Münch. med. Wochenschr., 1902. 1876, and Wien. Sitzungsber., **112**, Abt. II*b*, 1903; Monatsheft f. Chem., **25**; Hofmeister's Beiträge, **6**; see also foot-note 1, p. 33.

[5] Zanetti, Chem. Centralbl., 1898, I, p. 624; Eichholz, Journ. of Physiol., **23**.

[6] Hammarsten, l. c.; Hofmeister, Kauder and Pohl. Arch. f. exp. Path. u. Pharm., **20**.

Seralbumins are found in large quantities in blood-serum, blood-plasma, lymph, transudates, and exudates. Probably they also occur in other animal fluids and tissues. The proteids which pass into the urine under pathological conditions consist largely of seralbumin.

The seralbumin like the serglobulin seems also to be a mixture of at least two proteid bodies. The preparation of crystalline seralbumin (from horse-serum) was first performed by GÜRBER. It crystallizes with difficulty from other blood-sera (GRUZEWSKA). Even from horse-serum only a portion of the albumins is obtained as crystals, and it is also possible that the amorphous albumin, which is precipitated by ammonium sulphate with difficulty, represents two seralbumins (MAXIMOWITSCH). According to the statements of GÜRBER and MICHEL it would seem that the crystalline seralbumin is also a mixture, but this is disproved by the observations of SCHULZ, WICHMANN, and KRIEGER.[1] We know nothing as to the behavior of the amorphous fraction of the seralbumin in this regard. Because of the different coagulation temperatures, HALLIBURTON claims the existence of three different albumins in the blood-serum, a view which has been disputed by several experimenters and recently by HOUGARDY. On the other hand, the older investigations of KAUDER, as well as the more recent work of OPPENHEIMER,[2] seem to indicate a non-unit nature of the seralbumins, but this question is still an open one.

The crystalline seralbumin may perhaps be a combination with sulphuric acid (K. MÖRNER, INAGAKI). The coagulated albumin obtained from the aqueous solution of the crystals by the aid of alcohol has nearly the same elementary composition (MICHEL) as the amorphous mixture of albumin prepared from horse-serum (HAMMARSTEN and K. STARKE[3]). The average composition was C 53.06, H 6.98, N 15.99, S 1.84 per cent. K. MÖRNER, after the removal of the sulphuric acid from crystalline albumin, found 1.73 per cent total sulphur, which probably exists only as cystine. LANGSTEIN[4] has been able to split off a nitrogenous carbohydrate (glucosamine) from crystalline seralbumin. The quantity was so small that the question is still undecided whether or not the carbohydrate was not a contamination. The fact that ABDERHALDEN, BERGELL, and DÖRPINGHAUS[1] were able to prepare a seralbumin entirely free from carbohydrate and which did not respond to MOLISCH's very delicate reaction seems to be decisive on this point. The specific rotation of crystalline seralbumins from horse-serum was found by MICHEL to be $(\alpha)_D = -61\text{–}61.2°$ and by MAXIMOWITSCH on the contrary $(\alpha)_D = -47.47°$.

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[1] In regard to the literature on the crystalline seralbumins, see Schulz, Die Kristallisation von Eiweissstoffen, Jena, 1901; Maximowitsch, Maly's Jahresber., **31**, 35.

[2] Halliburton, Journ. of Physiol., **5** and **7**; Hougardy, Centrabl. f. Physiol., **15**, 665; Oppenheimer, Verhandl. d. physiol. Gesellsch., Berlin, 1902.

[3] Michel, Verhandl. d. phys.-med. Gesellsch. zu Würzburg, **29**, No. 3; K. Starke, Maly's Jahresber., **11**; K. Mörner, l. c.; Inagaki, Biochem. Centralbl., **4**, p. 515.

[4] K. Mörner, l. c.; Langstein, Hofmeister's Beiträge, **1**.

The crystalline and amorphous seralbumin in aqueous solution give the ordinary albumin reactions. The coagulation temperature of a 1 per cent solution poor in salts is about 50° C., but rises with the quantity of salt. The coagulation of the mixture of albumins from serum generally takes place at 70–85° C., but is essentially dependent upon the reaction and the amount of salt present. Up to the present time no seralbumin solution has been prepared free from mineral bodies. A solution as free from salts as possible does not coagulate either on boiling or on the addition of alcohol. On the addition of a little common salt it coagulates in both cases.[2]

Seralbumin differs from the albumin of the white of the hen's egg in the following particulars: It is more levogyrate; the precipitate formed by hydrochloric acid easily dissolves in an excess of the acid; it is rendered less insoluble by alcohol.

In preparing the seralbumin mixture, first remove the globulins, according to JOHANSSON, by saturating with magnesium sulphate at about 30° C. and filtering at the same temperature. The cooled filtrate is separated from the crystallized salt and is treated with acetic acid so that it contains about 1 per cent. The precipitate formed is filtered, pressed, dissolved in water with the addition of alkali to neutral reaction and the solution freed from salt by dialysis. The mixture of albumins may be obtained in a solid form from the dialyzed solution either by evaporating the solution at a gentle temperature or by precipitating with alcohol, which must be quickly removed. STARKE[3] has suggested another method, which is also to be recommended. The crystalline seralbumin may be prepared from serum freed from globulin by half saturating with ammonium sulphate, by the addition of more salt until a cloudiness occurs, and then proceeding according to the suggestion of GÜRBER and MICHEL. By acidification with acetic acid or sulphuric acid the crystallization may be considerably enhanced.[4] In the detection and quantitative estimation of seralbumin the filtrate from the globulin precipitated with magnesium sulphate can be heated to boiling, after acidification with a little acetic acid if necessary. The quantity of seralbumin is best calculated as the difference between the total proteins and the globulin.

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[1] Zeitschr. f. physiol. Chem., **41**.

[2] In regard to the relationship of neutral salts to heat coagulation, see J. Starke, Sitzungsber. d. Gesellsch. f. Morph. u. Physiol. in München, 1897.

[3] Johansson, Zeitschr. f. physiol. Chem., **9**; K. Starke, Maly's Jahresber., **11**.

[4] See Hopkins and Pinkus, Journ. of Physiol., **23**; Krieger, Über die Darstellung krystallinscher tierischer Eiweissstoffe, Inaug.-Dissert. Strassburg, 1899.

Summary of the elementary composition of the above-mentioned and described proteins (from horse-blood):

	C	H	N	S	O	
Fibrinogen............	52.93	6.90	16.66	1.25	22.26	(HAMMARSTEN)
Fibrin..............	52.68	6.83	16.91	1.10	22.48	"
Fibrin-globulin.........	52.70	6.98	16.06	....	.....	"
Serglobulin............	52.71	7.01	15.85	1.11	23.32	"
Seralbumin............	53.08	7.10	15.93	1.90	21.96	(MICHEL)

EMBDEN and KNOOP as well as LANGSTEIN have detected in blood-serum proteose-like substances which, according to them, occur preformed in the blood. NOLF has also found a small quantity of proteoses in the blood after an abundant absorption of proteoses by the intestine. According to ABDERHALDEN and OPPENHEIMER [1] the proteoses cannot be considered as normal blood constituents; even if we admit of their presence, the quantity is too small to be of any physiological importance.

v. BERGMANN and LANGSTEIN [2] have determined in dogs the residual nitrogen in the blood-serum, i.e., the nitrogen of the non-coagulable constituents. They found after feeding proteids that of the residual nitrogen 25 per cent existed as proteoses and 55 per cent as other products precipitable with phosphotungstic acid. In starving animals they found a maximum of 9 per cent of the residual nitrogen as proteoses.

The Blood-serum.

As above stated, the blood-serum is the clear liquid which is pressed out by the contraction of the blood-clot. It differs chiefly from the plasma in the absence of fibrinogen and in containing an abundance of fibrin ferment. Considered qualitatively, the blood-serum contains the same chief constituents as the blood-plasma.

Blood-serum is a sticky liquid which is more alkaline towards litmus than the plasma. The specific gravity in man is 1.027 to 1.032, average 1.028. The color is often strongly or faintly yellow; in human blood-serum it is pale yellow with a shade towards green, and in horses it is often amber-yellow. The serum is ordinarily clear; after a meal it may be opalescent, cloudy, or milky white, according to the amount of fat contained in the food.

Besides the above-mentioned bodies, the following constituents are found in the blood-plasma or blood-serum:

Fat occurs from 1-7 p. m. in fasting animals. After partaking of food the amount is increased to a great extent. *Soaps, cholesterin,* and *lecithin* are also found. Cholesterin occurs, according to HÜRTHLE,[3] at least in part, as fatty-acid esters (*serolin* according to BOUDET).

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[1] Embden and Knoop, Hofmeister's Beiträge, 3; Langstein, *ibid.*, 3; Nolf, Bull. Acad. Roy. Belg., 1903 and 1904; Abderhalden and Oppenheimer, Zeitschr. f. physiol. Chem., 42.

[2] v. Bergmann and Langstein, Hofmeister's Beiträge, 6.

[3] Zeitschr. f. physiol. Chem., 21, where Boudet is also cited. In regard to the quantity of these esters in bird-serum, see Brown, Amer. Journ. of Physiol., 2.

Sugar seems to be a physiological constituent of the plasma and serum According to the investigations of ABELES, EWALD, KÜLZ, v. MERING PAVY, SEEGEN, and MIURA[1] the sugar found is dextrose. STRAUSS[2] has also detected levulose in blood-serum and in transudates and exudates The question as to the occurrence of other varieties of sugar, such as isomaltose (PAVY and SIAU) and pentose (LÉPINE and BOULUD[3]), in blood-serum is still undecided. ASHER and ROSENFELD[4] have shown that at least a considerable part of the sugar can be removed from the blood by dialysis, hence it must exist in solution in the free state. These observations do not exclude the possibility of the existence of a part of the sugar in a combined form, as above stated (p. 180). Besides sugar the blood-serum contains, as first shown by J. OTTO, also another reducing non-fermentable substance. The statements of JACOBSEN, HENRIQUES, and BING,[5] that this substance is jecorin or lecithin sugar, do not have sufficient foundation. The nature of another carbohydrate in the blood, which is neither dextrorotatory nor reducing and which has been called *virtual sugar* by its discoverers, LÉPINE and BOULUD,[6] is also undetermined. The virtua sugar is more abundant in the blood of the right ventricle than in the arteria blood, and this in turn is richer than venous blood. In the passage of the blood through the lungs the virtual sugar is converted into ordinary sugar this may also occur in the capillaries of the greater circulatory system.

Conjugated glucuronic acids, which probably originate from the form elements, have been shown to occur in blood by the researches of P. MAYER LÉPINE and BOULUD.[7] The last two investigators find two definite glucuronic acids in the blood, both of which are levorotatory. One reduces FEHLING'S solution even at a temperature below 100°, while the other reduces it at above 100°. Such large amounts of the first acid often occur in the blood of dogs that the optical activity of the glucuronic acid counteracts that of the glucose. The second acid also occurs in larger quantities as compared with the sugar.

BERNARD[8] has shown that the quantity of sugar in the blood diminishes more or less rapidly on leaving the veins. LÉPINE, associated with BARRAL, has specially studied this decrease in the quantity of sugar and calls it *glycolysis*. LÉPINE and BARRAL, as well as ARTHUS, have shown that this glycolysis takes place in the complete absence of micro-organisms. It seems to be due to a soluble *glycolytic enzyme* whose activity is destroyed by heating to 54° C. This enzyme is derived, according to the above investigators, from the leucocytes and, according to LÉPINE,[1] has some connection with the pancreas. The glycolysis is, according to NASSE, RÖHMANN and SPITZER and SIEBER,[2] an oxidation which is produced, according to the two last-mentioned investigators, by an oxidation ferment. It is certainly not connected with the survival of the cells, but whether it is a vital or a post-mortem process is not decided.[3]

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[1] See v. Mering, Arch. f. (Anat. u.) Physiol., 1877 (this article contains numerous references); Seegen, Pflüger's Arch., **40**; Miura, Zeitschr. f. Biologie, **32**.

[2] Fortschritte d. Mediz., 1902.

[3] Pavy and Siau, Journ. of Physiol., **26**; Lépine et Boulud, Compt. rend., **133**, **135** and **136**.

[4] Centralbl. f. Physiol., **19**, p. 449.

[5] Otto, Pflüger's Arch., **35** (a good review of the older literature on sugar in the blood); Jacobsen, Centralbl. f. Physiol., **6**, 368; Henriques, Zeitschr. f. physiol. Chem. **23**; Bing, Skand. Arch. f. Physiol., **9**.

[6] Compt. rend., **137**.

[7] Mayer, Zeitschr. f. physiol. Chem., **32**; Lépine and Boulud, Compt. rend., **133**, **135**, **136**, **138**, **141**, and Journ. de Physiol., **7** (cited from Biochem. Centralbl., **4**, p. 421).

[8] Leçons sur le diabète, Paris, 1877.

The blood-plasma and the serum, as well as the lymph, also contain *enzymes* of various kinds. According to RÖHMANN, BIAL, HAMBURGER,[4] and others, *diastases*, which convert starch and glycogen into maltose or isomaltose, as well as a *maltoglucase* are found in the blood. HANRIOT has detected a *lipase* in the serum which decomposes butyrin, and which, according to him, decomposes neutral fats and other esters. The occurrence of a *butyrinase* is generally admitted, while the property of this lipase of splitting olein and other neutral fats is not generally acknowledged (ARTHUS, DOYON and MOREL [5]). This lipolytic property, if it exists to the extent that HANRIOT ascribes to it, must not be confounded with the transformation of fat into unknown substances soluble in water, a phenomenon first observed by COHNSTEIN and MICHAELIS and further studied by WEIGERT.[6] This property seems to be connected with the form-elements of the blood.

Besides the above-mentioned enzymes and thrombin, several other enzymes have been found in the blood-serum, namely, oxidases, catalases, proteolytic enzymes, rennin, and trypsin, and also the corresponding ant enzymes. We cannot enter into the discussion of these, nor of the man not chemically characterized bodies which have been called *toxines* an *antitoxines, immune bodies, alexines, hæmolysines, cytotoxines*, etc. It is als not within the scope of this book to discuss the *precipitines* which can b used as a biological reagent on account of their action upon various pr teins. It may be sufficient to state that the works of BORDET, EHRLIC WASSERMANN, SCHÜTZE, UHLENHAUT,[1] and others have shown that th repeated injection into an animal of a foreign protein body or of blood of different species of animal so changes the blood of this animal that it acquir precipitating properties towards the injected protein or blood. In th manner we obtain a biological reagent for various proteins and for blood different animals. This last behavior has become of great forensic impo tance, due to the work of UHLENHAUT. The various enzymes and ant enzymes, toxines and antitoxines, precipitines, etc., are as a rule precipitate with the globulin, but differ among each other in that some are carrie down by the euglobulin, while the others are carried down by the pseud globulin fraction.

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[1] In regard to the numerous memoirs of Lépine and Lépine et Barral, see Lyon médical., 62 and 63; Compt. rendus, 110, 112, 113, 120, and 139; Lépine, Le ferment glycolytique et la pathogénie du diabète (Paris, 1891), and Revue analytique et critique des travaux, etc., in Arch. de méd. expér. (Paris, 1892); Revue de médecine, 1895; Arthus, Arch. de Physiol. (5), 3, 4; Nasse and Framm, Pflüger's Arch., 63; Paderi, Maly's Jahresber., 26; see also Cremer, Physiologie des Glykogens in Ergebnisse d. Physiol., 1, Abt. 1.

[2] See Chapter I and N. Sieber, Zeitschr. f. physiol Chem., 39 and 44.

[3] See Arthus, l. c.; Colenbrander, Maly's Jahresber., 22; Rywosch, Centralbl. f. Physiol., 11, 495.

[4] Röhmann; Röhmann and Hamburger, Ber. d. deutsch. chem. Gesellsch., 25 and 27; Pflüger's Arch., 52 and 60; Bial, Ueber das diast. Ferm., etc., Inaug.-Diss. Breslau, 1892 (older literature). See also Pflüger's Arch., 52, 54, and 55.

[5] Hanriot, Compt. rend. soc. biol., 48 and 54; Compt. rend., 123 and 132; Arthus, Journ. de Physiol. et de Pathol., 4; Doyon and Morel, Compt. rend. soc. biol., 54; Achard and Clerç (Lipase in Disease), Compt. rend., 129, and Arch. d. med. expér., 14.

[6] Cohnstein and Michaelis, Pflüger's Arch., 65 and 69; Weigert, *ibid.*, 82.

Among the bodies which are found in the blood, and without doubt a met with in smaller or greater amounts in the plasma, are to be mentione *urea, uric acid* (found in human blood by ABELES), *phosphocarnic acid* (P NELLA[2]), *creatine, carbamic acid, paralactic acid, hippuric acid*, and traces *indol* (HERVIEUX[3]). Under pathological conditions the following bodi have been found: *xanthine bodies, leucine, tyrosine, lysine* (NEUBERG an RICHTER[4]), and *biliary constituents.*

The *coloring-matters* of the blood-serum are very little known. I equine blood-serum the biliary coloring-matter, bilirubin, besides other colo ing-matters, often occurs. The yellow coloring-matter of the serum seem to belong to the group of *luteins*, which are often called *lipochromes* or fa coloring matters. From ox-serum KRUKENBERG[5] was able to isolate wit amyl alcohol a so-called lipochrome whose solution shows two absorptio bands, of which one encloses the line F and the other lies between F and

The *mineral bodies* in serum and plasma are qualitatively, but n quantitatively, the same. A part of the calcium, magnesium, and pho phoric acid is removed on the coagulation of the fibrin. By means dialysis, the presence of sodium chloride, which forms the chief mass

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[1] The literature on this subject may be found in bacteriological journals and work See also L. Michaelis, Biochem. Centralbl., 3, p. 693.

[2] Abeles, Wien. med. Jahrb., 1887; Panella cited from Virchow's Jahresber. f. 190 150.

[3] Compt. rend. soc. biolog., 56.

[4] Deutsch. med. Wochenschr., 1904.

[5] Sitzungsber. d. Jen. Gesellsch. f. Med., 1885.

60-70 per cent of the total mineral bodies, lime-salts, sodium carbonate, and traces of sulphuric and phosphoric acids and of potassium, may be directly shown in the serum.[1] Traces of silicic acid, fluorine, copper, iron, manganese, and ammonia are claimed to have been found in the serum. As in most animal fluids, the chlorine and sodium are in the blood-serum in excess of the phosphoric acid and potassium (the occurrence of which in the serum is even doubted). The acids present in the ash are not sufficient to saturate the bases found, a condition which shows that a part of the bases is combined with organic substances, perhaps proteins. This coincides also with the fact that the great part of the alkalies does not exist in the serum as diffusible alkali compounds, carbonate and phosphate, but as non-diffusible compounds, protein combinations. According to Hamburger[2] 37 per cent of the alkali of the serum from horse-blood was diffusible and 63 per cent non-diffusible.

Iodine, which seems to be habitually found, is also considered as a mineral constituent of the plasma or serum (Gley and Bourcet), while arsenic, which is not found in all blood occurs only in human blood (Gautier, Bourcet[3]). Iodine occurs to a greater extent in menstrual blood than in other blood and does not exist as a salt, but as an organic compound (Bourcet).

The *gases* of the blood-serum, which consist chiefly of carbon dioxide with only a little nitrogen and oxygen, will be described when treating of the gases of the blood.

Because of the difficulty of obtaining plasma only a few analyses have been made. As an example the results of the analyses of the blood-plasma of the horse will be given below. The analysis No. 1 was made by Hoppe-Seyler.[4] No. 2 is the average of the results of three analyses made by Hammarsten. The figures are given for 1000 parts of the plasma.

	No. 1.	No. 2.
Water	908.4	917.6
Solids	91.6	82.4
Total proteins	77.6	69.5
Fibrin	10.1	6.5
Globulin	.....	38.4
Seralbumin	.....	24.6
Fat	1.2	12.9
Extractive substances	4.0	
Soluble salts	6.4	
Insoluble salts	1.7	

Lewinsky[5] has determined the total proteins and the individual proteins in the blood-plasma of man and animals with the following results.

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[1] See Gürber, Verhandl. d. phys.-med. Gesellsch. zu Würzburg, **23**.

[2] In regard to method, see Arch. f. (Anat. u.) Physiol., 1898.

[3] Gley et Bourcet, Compt. rend., **130**; Bourcet, *ibid.*, **131**; Gautier, *ibid.*, **131**.

[4] Cit. from v. Gorup-Besanez's Lehrbuch der physiol. Chem., 4. Aufl., 346.

[5] Pflüger's Arch., **100**.

	Total Protein.	Albumin.	Globulin.	Fibrinogen.
Man	72.6	40.1	28.3	4.2
Dog	60.3	31.7	22.6	6.0
Sheep	72.9	38.3	30.0	4.6
Horse	80.4	28.0	47.9	4.5
Pig	80.5	44.2	29.8	6.5

ABDERHALDEN has made complete analyses of the blood-serum of several domestic animals. From these analyses as well as from those made by HAMMARSTEN of the serum from human, horse, and ox blood it follows that the amount of solids ordinarily varies between 70–97 p. m. The chief mass of the solids consists of proteins, about 55–84 p. m. In hens HAMMARSTEN found much lower values, namely, 54 p. m. solids, with only 39.5 p. m. protein, and HALLIBURTON found only 25.4 p. m. protein in frog's blood. The relationship between globulin and seralbumin is, as shown by the analyses of HAMMARSTEN, HALLIBURTON, and RUBBRECHT,[1] very different for various animals, but may also vary considerably in the same species of animal. In human blood-serum HAMMARSTEN found more seralbumin than globulin, and the relationship of serglobulin to seralbumin was as 1 : 1.5. LEWINSKY found the relationship in man greater than 1, indeed 1 : 1.39–2.13. In regard to the quantity of the remaining organic constituents of the serum we refer the reader to ABDERHALDEN's complete analyses.

In starvation it seems, as first found by BURCKHARDT and recently substantiated by GITHENS,[2] that the quantity of globulin relative to that of albumin is increased. A change in the relationship with a decrease in the albumin and increase in the globulin may also occur in animals which have been made sick or in part immune by inoculation with pathogenic microorganisms (LANGSTEIN and MAYER[3]). The total protein content is raised in nearly all cases. The amount of fibrinogen in the plasma is especially increased by pneumococci, streptococci, and pus-staphylococci (P. MÜLLER[4]).

The quantity of mineral bodies in the serum has been determined by many investigators. The conclusion drawn from the analyses is that there exists a rather close correspondence between human and animal blood-serum, and it is therefore sufficient to give here the analysis of C. SCHMIDT[5] of (1) human blood, and BUNGE and ABDERHALDEN's analyses (2) of serum of ox, bull, sheep, goat, pig, rabbit, dog, and cat. The results correspond to 1000 parts by weight of the serum.

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[1] Abderhalden, Zeitschr. f. physiol. Chem., **25**; Hammarsten, Pflüger's Arch., **17**; Halliburton, Journ. of Physiol., **7**; Rubbrecht, Travaux du laboratoire de l'institut de physiologie de Liège, **5**, 1896.

[2] Burckhardt, Arch. f. exp. Path. u. Pharm., **16**; Githens, Hofmeister's Beiträge, **5**.

[3] Hofmeister's Beiträge, **5**.

[4] *Ibid.*, **6**.

[5] Cit. from Hoppe-Seyler, Physiol. Chem., 1881, p. 439.

	1	2
K_2O	0.387–0.401	0.226–0.270
Na_2O	4.290–4.290	4.251–4.442
Cl	3.565–3.659	3.627–4.170
CaO	0.155–0.155	0.119–0.131
MgO	0.101	0.040–0.046
P_2O_5 (inorg.)		0.052–0.085

Even if we bear in mind that certain bodies, such as carbon dioxide, are driven off during incineration and that other bodies, such as sulphuric acid and phosphoric acid, are formed from sulphurized and phosphorized organic substances, still quantitative analyses like the above are not sufficient for the scientific demands of to-day. They do not show the true composition and especially do not give an explanation of the number of different ions present in the serum or in other fluids, a question which is of the greatest physiological importance. An answer to these questions is obtainable only by physico-chemical investigations, which have thus far been used chiefly in determining the molecular concentration, the amount of electrolytes and non-electrolytes, and the degree of dissociation.

The molecular, or, as Hamburger calls it, the osmotic concentration which gives the total number of molecules and ions in the litre, is measured by the osmotic pressure, and it may be expressed by $\frac{\Delta}{1.85}$ if we make use of the depression of the freezing-point (Δ) instead of the osmotic pressure, as a gram-molecule of a non-electrolyte, or an equivalent number of ions, when in 1 litre of solution, causes a depression of the freezing-point of 1.85°.

The average depression of the freezing-point of human blood-serum is ordinarily given as $\Delta = -0.526^\circ$. According to Th. Cohn[1] the actual depression of the freezing-point of normal human blood is $\Delta = -0.537^\circ$. This freezing-point depression, it seems, is a little lower than that of the sera of other mammals that have been investigated: -0.560° (horse) to 0.619° (sheep). The molecular concentration of the blood-serum of various mammals also differs only slightly in each case, according to Bugarsky and Tangl,[2] and amounts on an average to about 0.320 mol per litre. The average freezing-point depression corresponds closely to that of a common salt solution of 9 p. m. ($\Delta = -0.551^\circ$ to -0.561°), and at present such a solution is considered as a physiological salt solution for man and other mammals.

The conditions are otherwise with sea-animals which live in a medium rich in salts. According to Bottazzi the blood (or the fluid of the cavities) of invertebrate sea-animals has an osmotic pressure which corresponds to an average freezing-point depression of $\Delta = -2.29^\circ$, i.e., exactly the same as the sea-water in which they live. In the cartilaginous fishes nearly the same conditions exist, while in the Teleostei the osmotic pressure is much lower than that of the sea-water, but is about one half greater than the blood of land-vertebrates. The Teleostei are the first in the scale of development of animals to show an independence of the osmotic pressure of the inner fluids from the surrounding media.

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[1] Mitteil. aus d. Grenzgeb. d. Mediz. u. Chir., 15.

[2] In regard to the literature on this subject we refer to Hamburger, Osmotischer Druck und Ionenlehre, from which the author obtained most of the facts given. See also Höber, Physikalische Chemie der Zelle und der Gewebe, 2. Aufl., 1906.

The researches of FREDERICQ[1] have led to the same results. In the sea-invertebrates examined the blood (the hæmolymph) had the same molecular concentration and same salt content as the exterior medium. In plagiostoma the blood had, with equal molecular concentration, a considerably lower salt content than the sea-water. The equality of the molecular concentration was maintained in these cases by a high urea content. In all bony fishes of salt and fresh waters and in fresh-water crabs the blood differs markedly in regard to molecular concentration, as well as in salt content, from the exterior medium.

There are recorded a great number of investigations on the changes in the osmotic pressure or the molecular concentration of the blood-serum under various physiological conditions as well as in disease, but still it is no doubt too early to draw any definite conclusions from these observations.

As seen from the above, blood-serum contains electrolytes as well as non-electrolytes. Of the latter the proteins and also sugar, fat, lecithin, urea, and the so-called extractive bodies are of the greatest importance. The electrolytes comprise the various ions and the undissociated molecules of the salts of the serum. The electrolytes are the only constituents of the serum which conduct the electric current, while the non-electrolytes retard the conductivity. The degree of dissociation can also be calculated from the determination of the conductivity of the blood-serum.

The coefficient of dissociation is, according to ARRHENIUS, the relationship between the number of ions in a solution and the number of ions which would be present if the electrolytes were completely dissociated. As the conductivity of a solution of electrolytes is determined by the number of ions (admitting that the migration velocity of the ions is the same for different dilutions), the above coefficient α can be calculated by the formula $\alpha = \frac{\lambda_v}{\lambda_\infty}$. In this formula λ_v represents the conductivity of the original dilution (i.e., of the undiluted serum) and λ_∞ the conductivity of the completely dissociated molecules (ions) after sufficiently strong dilution of the serum with water.

According to the above principle the degree of dissociation of serum has been determined by several investigators, especially BUGARSKY and TANGL, OKER-BLOM, and VIOLA. This last investigator found that the degree of dissociation of the blood-serum of healthy human beings was equal to 0.68–0.73. According to HAMBURGER the results thus obtained experimentally must be a little too low for certain reasons, and we therefore can consider the dissociation coefficient to be between 0.65 and 0.82.

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[1] Arch. de Biol., 20. Cited from Centralbl. f. Physiol., 19, p. 21.

As above stated, the non-electrolytes have a retarding action upon the conductivity, and according to **Bugarsky** and **Tangl** each gram of protein in 100 c.c. of serum diminishes the electrical conductivity of the serum about 2.5 per cent. By making use of this fact, the corrected conductivity of the electrolytes present can be determined from the conductivity. The corrected conductivity is partly dependent upon the chlorides and partly upon the other salts (which are nearly identical with the quantity of Na_2CO_3). If the amount of NaCl of the serum is determined by analysis we can calculate the conductivity of the other salts by subtracting the calculated conductivity of a solution of NaCl of similar concentration (which can be done according to **Kohlrausch**'s method) from the total corrected conductivity. From these results we can calculate the molecular concentration of the chlorides and of the non-chlorides. The sum of these two is subtracted from the molecular concentration of the serum, when the molecular concentration of the non-electrolytes is obtained.

Bugarsky and **Tangl** have made physico-chemical analyses of blood-serum of certain mammals according to the principle given above. They found that the molecular concentration was, on an average, about 0.320 mol per litre, that about three fourths of the total dissolved molecules of blood-serum were electrolytes, although the serum contained about 70-80 p. m. proteid and 10 p. m. inorganic bodies, and also that three fourths of the quantity of electrolytes consisted of NaCl. **Viola** and **Bousquet** have recorded less complete osmotic chemical analyses of blood-serum of diseased and healthy human beings, making use of methods somewhat different in principle.

In the determination of the alkalinity of blood and blood-serum, up to the present time we have estimated the amount of alkali by titration with an acid. We cannot dispense with such determinations, although they do not yield any information as to the true alkalinity, apart from the fact that the results are dependent upon the indicator used, because we understand as true alkalinity the concentration of the hydroxyl ions. The Na_2CO_3 is in aqueous solution more or less dissociated into $2Na^+$ and $CO_3^=$, depending upon the dilution. The $CO_3^=$ ions combine partly with the H^+ ions of the dissociated water, forming HCO_3^-, and the corresponding HO^- ions produce the alkaline reaction. If now, by the addition of a little acid, a few of the HO^- ions are removed, then the equilibrium is disturbed, a new quantity of Na_2CO_3 is dissociated, and this process is repeated every time a new quantity of acid is added until all the carbonate is dissociated. The dissociation of the carbonate existing in the original concentration, upon which the number of HO^- ions is dependent, cannot therefore be determined by titration. For these reasons **Höber** has worked out a physico-chemical method of determining alkalinity, based upon **Nernst**'s theory of liquid chains. This method was used later by **Farkas**, **Fränckel**, and **Höber** after a few changes. The investigations of these last-mentioned experimenters show that the concentration of the hydroxyl ions in blood-serum and blood is nearly the same as in distilled water, and that these fluids are nearly neutral in behavior, which fact is caused by the presence of carbonic acid. FRIEDENTHAL,[1] by testing serum with phenolphthalein, arrived at similar results.

II. THE FORM-ELEMENTS OF THE BLOOD.

The Red Blood-Corpuscles.

The blood-corpuscles are round, biconcave disks without membrane and nucleus in man and mammalia (with the exception of the llama, the camel, and their congeners). In the latter animals, as also in birds, amphibia, and fishes (with the exception of the Cyclostoma), the corpuscles have in general a nucleus, are biconvex and more or less elliptical. The size varies in different animals. In man they have an average diameter of 7 to 8 μ (μ=0.001 mm.) and a maximum thickness of 1.9 μ. They are heavier than the blood-plasma or serum, and therefore sink in these liquids. In the discharged blood they may lie sometimes with their flat surfaces together, forming a cylinder like a roll of coin (rouleaux). The reason for this phenomenon, which is considered as an agglutination, has not been sufficiently studied, but as it may be observed in defibrinated blood it seems probable that the formation of fibrin has nothing to do with it.

The number of red blood-corpuscles is different in the blood of various animals. In the blood of man there are generally 5 million red corpuscles in 1 c.mm., and in woman 4 to 4.5 million.

The blood-corpuscles consist essentially of two chief constituents, the stroma, which forms the real protoplasm, and the intraglobular contents, whose chief constituent is hæmoglobin. We cannot state anything positive for the present in regard to a more detailed arrangement, and the views on this subject are somewhat divergent. The two following views are more or less related to each other. According to one view the blood-corpuscles consist of a membrane which encloses a hæmoglobin solution, while the other view considers the stroma as a protoplasmic structure soaked with hæmoglobin. This latter view is in accord with the assumption as to an outside boundary-layer.

Thus according to HAMBURGER the stroma forms a protoplasmic net in whose meshes there exists a red fluid or semi-fluid mass which consists in great measure of hæmoglobin. This mass represents the water-attracting force of the blood-corpuscles, and besides this it is also considered that the outer protoplasmic boundary is semi-permeable, i.e., permeable to water but not permeable to certain crystalloids. The researches of KÖPPE,

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[1] Höber, Pflüger's Arch., 81 and 99; Farkas, see Biochem. Centralbl., 1, 626; Fränckel, Pflüger's Arch., 96; Friedenthal, Zeitschr. f. allg. Physiol., 1 and 4.

ALBRECHT, PASCUCCI, RYWOSCH,[1] and others indicate the presence of a special envelope or boundary-layer, and there is no doubt that the outer layer contains so-called lipoids, such as cholesterin, lecithin, and similar bodies.

The red blood-corpuscles retain their volume in a salt solution which has the same osmotic pressure as the serum of the same blood, although they may change their form in such solutions, becoming more spherical, and may also undergo a chemical change (HAMBURGER, HEDIN, and others). Such a salt solution is *isotonic*[2] with the blood-serum, and its concentration for a NaCl solution is approximately 9 p. m. for human and mammalian blood. A solution of greater concentration, a *hyperisotonic* solution, abstracts water from the blood-corpuscles until osmotic equilibrium is established, hence the corpuscles shrink and their volume becomes smaller. In solutions of less concentration, *hypisotonic* solutions, the corpuscles swell up, due to the taking up of water, and this swelling may be so great, as on diluting the blood with water, that the hæmoglobin is separated from the stroma and passes into the watery solution. This process is called *hæmolysis.*

A hæmolysis may also be brought about by alternately freezing and thawing the blood, as well as by the action of various chemical substances, which act as protoplasmic poisons. These bodies are ether, chloroform, alkalies, bile-acids, solanin, saponin, and also the saponin substances, which have a very strong hæmolytic action. Of special interest in this regard are the hæmolysines, which act like toxines. These hæmolysines may be metabolic products of bacteria and may be formed by higher plants and by animals, such as snakes, toads, bees, spiders, and others. Finally, the hæmolysines or globulicidal bodies, occurring normally in blood-sera or produced in the immunization of the blood, also belong here.

It seems that hæmolysis is brought about in various cases in different ways. In the hæmolysis by means of water we are probably dealing with a destruction or rupture of the boundary-layer, while such bodies as ether, chloroform, alkalies, bile-acids, and saponin substances, which dissolve lipoids or form combinations therewith, in this way cause the passage of the hæmoglobin to the outside (KÖPPE, RANSOM and KOBERT, PESKIND, PASCUCCI). The action of other hæmolysines, such as snake-venom and tetanotoxine, seems to be an action connected with the lecithin (KYES, PASCUCCI[3]).

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[1] See Hamburger, Osmotischer Druck und Ionenlehre, 1902; Köppe, Pflüger's Arch., **99** and **107**; Albrecht, Centralbl. f. Physiol., **19**; Pascucci, Hofmeister's Beiträge, **6**; Rywosch, Centralbl. f. Physiol., **19**.

[2] The work of Hamburger, Hedin, Eykman, Köppe, and others on isotonism, and the literature on this subject, may be found in Hamburger, Osmotischer Druck und Ionenlehre, 1902.

[3] Köppe, l. c.; Peskind, Amer. Journ. of Physiol., **12**; Ransom and Kobert, cited by Pascucci, Hofmeister's Beiträge, **6**; Kyes, Zeitschr. f. physiol. Chem., **41**, and Berl. klin. Wochenschr., 1904.

When the hæmoglobin is separated from the so-called stroma by a sufficiently strong dilution with water the stroma is found in the solution in a swollen condition. By the action of carbon dioxide, by the careful addition of acids, acid salts, tincture of iodine, or certain other bodies, this residue, rich in proteins, condenses, and in many cases the form of the blood-corpuscles may be again obtained. This residue, the so-called ghosts or stromata of the blood-corpuscles, can also be directly colored in dilute blood by methyl violet and in this way detected (KÖPPE), and attempts have been made to isolate it for chemical investigation. In the following pages we mean by the name stroma only that residue that remains after the removal of hæmoglobin and other bodies soluble in water.

To isolate the stromata from the blood-corpuscles, they are washed first by diluting the blood with 10–20 vols. of a 1–2 per cent common salt solution and then separating the mixture by centrifugal force or by allowing it to stand at a low temperature. This is repeated a few times until the blood-corpuscles are freed from serum. These purified blood-corpuscles are, according to WOOLDRIDGE, mixed with 5–6 vols. of water, and then a little ether is added until complete solution is obtained. The leucocytes gradually settle to the bottom, a movement which may be accelerated by centrifugal force, and the liquid which separates therefrom is very carefully treated with a 1 per cent solution of $KHSO_4$ until it is about as dense as the original blood. The separated stromata are collected on a filter and quickly washed. PASCUCCI,[1] on the contrary, treats the mass of corpuscles with 15–20 vols. of a $\frac{1}{5}$ saturated ammonium-sulphate solution, allows the corpuscles to settle, siphons off the fluid, repeatedly centrifuges, allows the residue to dry quickly (on porcelain plates) at the ordinary temperature, and then washes with water until the blood-pigments and the other soluble bodies are dissolved out.

WOOLDRIDGE found as constituents of the stromata *lecithin*, *cholesterin*, *nucleoalbumin*, and a *globulin* which, according to HALLIBURTON, is probably a nucleoproteid which he calls *cell-globulin*. No nuclein substances or seralbumin or proteoses could be detected by HALLIBURTON and FRIEND. According to PASCUCCI, the stromata (from horse-blood) consists of $\frac{1}{3}$ cholesterin and lecithin (besides a little cerebroside), and $\frac{2}{3}$ protein substances and mineral bodies. The nucleated red blood-corpuscles of the bird contain, according to PLÓSZ and HOPPE-SEYLER,[2] *nuclein* and a protein which swells to a slimy mass in a 10 per cent common salt solution, and which seems to be closely related to the hyaline substance (*hyaline substance* of ROVIDA, see page 141) occurring in the lymph-cells. In the mass extracted by alcohol from the blood-corpuscles of the hen,

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[1] Hofmeister's Beiträge, 6.

[2] Wooldridge, Arch. f. (Anat. u.) Physiol., 1881, 387; Halliburton and Friend, Journal of Physiol., 10; Halliburton, *ibid.*, 18; Plósz, Hoppe-Seyler's Med. chem. Untersuch., 510.

ACKERMANN[1] found 3.93 per cent phosphorus and 17.2 per cent nitrogen, which on calculation gave 42.10 per cent nucleic acid and 57.82 per cent histone. The non-nucleated red blood-corpuscles are, as a rule, very poor in protein, but are rich in hæmoglobin; the nucleated corpuscles are richer in protein and poorer in hæmoglobin than the non-nucleated.

A gelatinous, fibrin-like protein body may be obtained from the red blood-corpuscles under certain circumstances. This fibrin-like mass has been observed on freezing and then thawing the sediment of the blood-corpuscles, or on discharging the spark from a large Leyden jar through the blood, or on dissolving the blood-corpuscles of one kind of animal in the serum of another (LANDOIS, *stroma-fibrin*); i.e., in the so-called *hæm-agglutination*, a clumping of the red blood-corpuscles into clusters takes place. This agglutination can be brought about by bodies similar to the hæmolysines and also by serum constituents produced normally or by immunization. It has not been shown that a fibrin formation from the stroma takes place. Fibrinogen has only been detected in the red corpuscles of frogs' blood (ALEX. SCHMIDT and SEMMER[2]).

Closely related to the anatomical and chemical structure of the erythrocytes is the question which is important, for the metabolism in the blood, as to the permeability of the erythrocytes, that is, their power of taking up substances of different kinds. On this subject we have the researches of GRÜNS, EYKMAN, OVERTON, KÖPPE, and especially those of HAMBURGER and his collaborators, and of HEDIN.[3] As a result of these researches, it has been shown that the blood-corpuscles are completely impermeable for the ordinary varieties of sugar, for arabite and mannite, and, as it appears, also for the cations Ca^{++}, Sr^{++}, Ba^{++}, Mg^{++}. On the other hand, they are permeable for NH_4^+ ions, as also for acids and alkalies.[4] They are also permeable for alcohols (more readily the fewer hydroxyl groups the molecule contains), aldehydes (with the exception of paraldehyde), ketones, ethers, esters, urea, bile salts, and other compounds. They are only slightly permeable for amino-acids. Towards the neutral potassium and sodium salts, according to KÖPPE and HAMBURGER, the blood-corpuscles are impermeable for the cations K^+ and Na^+, and permeable, on the contrary, for the anions when an exchange of an anion, for example CO_3^-, in the blood-corpuscles is possible with an anion in the outer fluid, for example with Cl^-, Br^-, NO_3^-, etc. HÖBER[5] has further shown that the blood-corpuscles are permeable for anions under the influence of carbon dioxide. Such an exchange of ions can be especially observed, according to HAMBURGER, in the erythrocytes suspended in NaCl solution and treated with CO_2, when the outer fluid becomes alkaline, due to the formation of Na_2CO_3 by the migration of Cl^- ions into the corpuscles and an outward migration of the $CO_3^{=}$ ions. For every one bivalent $CO_3^{=}$ ion there must migrate inward two univalent Cl^- ions; but as every ion irrespective of whether it is uni- or bivalent has the same osmotic pressure, therefore the osmotic pressure of the blood-corpuscles must be raised, and hence a swelling up takes place, due to their taking up water. The question as to how far these observations can be applied to the blood-corpuscles in their serum, i.e., to the blood, requires further proofs.[1]

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[1] Zeitschr. f. physiol. Chem., 43.

[2] Landois, Centralbl. f. d. med. Wissensch., 1874, 421; Schmidt, Pflüger's Arch., 11, 550–559.

[3] In regard to the literature, see Hamburger, Osmotischer Druck- und Ionenlehre.

[4] See Höber, Pflüger's Arch., 101 and 102.

[5] Pflüger's Arch., 102

The *mineral bodies* of the red corpuscles will be treated in connection with their quantitative constitution.

The constituent of the blood-corpuscles existing in greatest quantity is the red pigment hæmoglobin.

Blood-pigments.

According to HOPPE-SEYLER the coloring-matter of the red blood-corpuscles is not in a free state, but combined with some other substance. The crystalline coloring-matter, the hæmoglobin or oxyhæmoglobin, which may be isolated from the blood, is considered, according to HOPPE-SEYLER, as a cleavage product of this compound, and it acts in many ways unlike the questionable compound itself. This compound is insoluble in water and uncrystallizable. It strongly decomposes hydrogen peroxide without being oxidized itself; it shows a greater resistance to certain chemical reagents (as potassium ferricyanide) than the free coloring-matter; and, lastly, it gives off its loosely combined oxygen much more easily in vacuum than the free pigment. To distinguish between the cleavage products, the hæmoglobin and the oxyhæmoglobin, HOPPE-SEYLER calls the compound of the blood-coloring matter of the venous blood-corpuscles *phlebin*, and that of the arterial *arterin*. Other investigators, such as H. U. KOBERT and BOHR,[2] the latter calling the pigment of the blood-corpuscles *hæmochrom*, are of a similar opinion. Since the above-mentioned combinations of the blood-coloring matters with other bodies, for example (if they really do exist) with lecithin, have not been closely studied, the following statements will apply only to the free pigment, the hæmoglobin.

The color of the blood depends in part on *hæmoglobin* and in part on a molecular combination of this substance with oxygen, the *oxyhæmoglobin*.

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[1] See Petry, Hofmeister's Beiträge, 3.

[2] Hoppe-Seyler, Zeitschr. f. physiol. Chem., 13, 479; H. U. Kobert, Das Wirbeltierblut in mikro-kristallogr. Hinsicht, Stuttgart, 1901; Bohr, Centralbl. f. Physiol., 17, p. 688.

We find in blood after asphyxiation almost exclusively hæmoglobin, in arterial blood disproportionately large amounts of oxyhæmoglobin, and in venous blood a mixture of both. Blood-coloring matters are found also in striated as well as in certain smooth muscles, and lastly in solution in different invertebrates. The quantity of hæmoglobin in human blood may indeed be somewhat variable under different circumstances, but amounts to about 14 per cent on an average, or 8.5 grams for each kilo of the weight of the body.

Hæmoglobin belongs to the group of compound proteids and yields as cleavage products, besides very small amounts of volatile fatty acids and other bodies, chiefly a protein *globin* and a coloring-matter, *hæmochromogen* (about 4 per cent), containing iron, which in the presence of oxygen is easily oxidized into *hæmatin.*

As first shown by SCHUNCK and MARCHLEWSKI, and especially by the work of the latter, a close relationship exists between chlorophyll and the blood-pigment, because a derivative of the first, phylloporphyrin, stands very close in certain regards to a derivative of the blood-pigment hæmatoporphyrin. By the investigations of NENCKI in conjunction with MARCHLEWSKI and ZALESKI,[1] it was shown that hæmopyrol could be prepared from the derivatives of both the leaf-pigment and the blood-pigments by reduction. The fact that chlorophyll and blood-pigments are closely related and are constructed from the same mother-substance is of the greatest biological importance.

The hæmoglobin prepared from different kinds of blood has not exactly the same composition, which seems to indicate the presence of different hæmoglobins. The analyses by different investigators of the hæmoglobin from the same kind of blood do not always agree with one another, which probably depends upon the somewhat varying methods of preparation. The following analyses are given as examples of the constitution of different hæmoglobins:

Hæmoglobin from the	C	H	N	S	Fe	O	P_2O_5	
Dog............	53.85	7.32	16.17	0.390	0.430	21.84	....	(HOPPE-SEYLER)
"	54.57	7.22	16.38	0.568	0.336	20.93	....	(JAQUET)
Horse..........	54.87	6.97	17.31	0.650	0.470	19.73	....	(KOSSEL)
"	51.15	6.76	17.94	0.390	0.335	23.43	....	(ZINOFFSKY)
Ox............	54.66	7.25	17.70	0.447	0.400	19.543	....	(HÜFNER)
Pig............	54.17	7.38	16.23	0.660	0.430	21.360	....	(OTTO)
"............	54.71	7.38	17.43	0.479	0.399	19.602	....	(HÜFNER)
Guinea-pig.......	54.12	7.36	16.78	0.580	0.480	20.680	....	(HOPPE-SEYLER)
Squirrel.........	54.09	7.39	16.09	0.400	0.590	21.440	....	"
Goose...........	54.26	7.10	16.21	0.540	0.430	20.690	0.770	"
Hen...........	52.47	7.19	16.45	0.857	0.335	22.500	0.197	(JAQUET)

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[1] Schunck and Marchlewski, Annal. d. Chem. u. Pharm., 278, 284, 288, 290; Nencki, Ber. d. deutsch. chem. Gesellsch., 29; Marchlewski and Nencki, Ber. d. d. chem. Gesellsch.. 34; Nencki and Zaleski, *ibid.*; Marchlewski, Chem. Centralbl., 1902, I. 1016; Zaleski, Zeitschr. f. physiol. Chem., 37.

The question whether the amount of phosphorus in the hæmoglobin from birds exists as a contamination or as a constituent has not been decided. According to Inoko the hæmoglobin from goose-blood consists of a combination between nucleic acid and hæmoglobin. In the hæmoglobin from the horse (Zinoffsky), the pig, and the ox (Hüfner) we have 1 atom of iron to 2 atoms of sulphur, while in the hæmoglobin from the dog (Jaquet) the relation is 1 to 3. From the data of the elementary analysis, as also from the amount of loosely combined oxygen, Hüfner[1] has calculated the molecular weight of dog-hæmoglobin as 14 129 and the formula $C_{636}H_{1025}N_{164}FeS_3O_{181}$. According to the more recent determinations of Hüfner and Jaquet,[2] ox-hæmoglobin contains an average of 0.336 per cent iron, from which a molecular weight of 16 669 may be calculated. The hæmoglobin from various kinds of blood not only shows a diverse constitution, but also a different solubility and crystalline form, and a varying quantity of water of crystallization; hence we infer that there are several kinds of hæmoglobin. Bohr is a very zealous advocate of this supposition. He has been able to obtain hæmoglobins from dog- and horse-blood, by fractional crystallization, which had different powers of combining with oxygen and contained different quantities of iron. Hoppe-Seyler had already prepared two different forms of hæmoglobin crystals from horse-blood, and Bohr concludes from all these observations that the ordinary hæmoglobin consists of a mixture of different hæmoglobins. In opposition to this statement, Hüfner[3] has shown that only one hæmoglobin exists in ox-blood, and that this is probably true for the blood of many other animals.

Oxyhæmoglobin, which has also been called hæmatoglobulin or hæmatocrystallin, is a molecular combination of hæmoglobin and oxygen. For each molecule of hæmoglobin 1 molecule of oxygen is present; and the amount of loosely combined oxygen which is united to 1 gram of hæmoglobin (of the ox) has been determined by Hüfner[4] as 1.34 c.c. (calculated at 0° C. and 760 mm. mercury).

According to Bohr, the facts are different. He differentiates between four oxyhæmoglobins, according to the quantity of oxygen which they absorb, namely, α-, β-, γ-, and δ-oxyhæmoglobin, all having the same absorption-spectrum and 1 gram combining with respectively 0.4, 0.8, 1.7, and 2.7 c.c. oxygen at the temperature of the room and with an oxygen pressure of 150 mm. mercury. The γ-oxyhæmoglobin is the ordinary one obtained by the customary method of preparation. BOHR designates as α-oxyhæmoglobin the crystalline powder obtained by drying γ-oxyhæmoglobin in the air. On dissolving α-oxyhæmoglobin in water it is converted into β-oxyhæmoglobin without decomposition, and the quantity of iron is increased. On keeping a solution of γ-oxyhæmoglobin in a sealed tube it is transformed into δ-oxyhæmoglobin, although the exact conditions under which this change takes place are not known. According to HÜFNER[1] these are nothing but mixtures of genuine and partly decomposed hæmoglobins.

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[1] Hoppe-Seyler, Med. chem. Untersuch., 370; Jaquet, Zeitschr. f. physiol. Chem., 14, 296; Kossel. *ibid.*, 2, 150; Zinoffsky, *ibid.*, 10; Hüfner, Beitr. z. Physiol., Festschr. f. C. Ludwig, 1887, 74–81, Journ. f. prakt. Chem. (N. F.), 22; Otto, Zeitschr. f. physiol. Chem., 7, Inoko, *ibid.*, 18.

[2] Arch. f. (Anat. u.) Physiol., 1894.

[3] Bohr, "Sur les combinaisons de l'hémoglobine avec l'oxygène," Extrait du Bulletin de l'Académie Royale Danoise des sciences, 1890; also Centralbl. f. Physiol., 1890, 249. Hoppe-Seyler, Zeitschr. f physiol. Chem., 2; Hüfner, Arch. f. (Anat. u.) Physiol., 1894.

[4] Arch. f. (Anat u.) Physiol., 1901, Suppl.

The ability of hæmoglobin to take up oxygen seems to be a function of the iron it contains, and when this is calculated as about 0.33–0.40 per cent, then 1 atom of iron in the hæmoglobin corresponds to about 2 atoms or 1 molecule of oxygen. By increasing the partial pressure as well as by increasing the quantities of oxygen, the hæmoglobin in solution takes up more oxygen, until it is completely saturated, when 1 molecule of hæmoglobin is combined with 1 molecule of oxygen. Still this reaction is reversible according to the type $1(\mathrm{Hb})+1(\mathrm{O}_2)\rightleftarrows 1(\mathrm{OHb})$, and with diminished oxygen pressure a dissociation must take place with the giving up of oxygen and a re-formation of hæmoglobin. The equilibrium between oxyhæmoglobin, hæmoglobin, and oxygen is determined according to the law of mass-action, and according to the investigations of HÜFNER it is possible to calculate the relationship between oxyhæmoglobin (OHb) and hæmoglobin (Hb), at every desired partial pressure of the oxygen, by a formula suggested by him. According to BOHR[2] this formula does not have sufficient basis and does not correspond to the facts. BOHR found, in opposition to HÜFNER'S statements, that with the same oxygen tension the absorption of oxygen by a hæmoglobin solution changes with the concentration, and that a dilute solution combines with more oxygen, calculated per 1 gram hæmoglobin, than a concentrated solution. BOHR suggested another formula expressing the relationship between the oxygen absorption and the oxygen tension, based upon the assumption that, besides the dissociation of the oxygen-hæmoglobin compound, a dissociation of the hæmoglobin into a part containing iron and a part not containing iron also takes place. This formula, which in fact accords well with BOHR'S findings, is nevertheless only true for a hæmoglobin solution and not for blood, as, according to BOHR, the blood-pigment in the blood-corpuscles (the hæmochrom) is changed on being converted into hæmoglobin. HENRI also finds that HÜFNER'S formula for the dissociation of oxyhæmoglobin is not useful, basing his claim upon theoretical considerations and upon unfinished investigations.

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[1] Arch. f. (Anat. u.) physiol., 1894.

[2] Bohr, Centralbl. f. Physiol., 17, pp. 682 and 688; Henri, Compt. rend. soc. biolog., 56.

The native pigment, the hæmochrom, combines, according to **Bohr**, in maximo with the same quantity of oxygen as the corresponding hæmoglobin, when the latter is prepared without the use of means having a strong action; still from this it does not follow that the oxygen combination in hæmochrom is identical with that in hæmoglobin. According to **Bohr** this is not the case, at least with diminished pressure, for with low oxygen tension more oxygen is taken up by the blood than by a corresponding hæmoglobin solution. The curve showing the oxygen absorption is lower in this case for a hæmoglobin solution than for blood. The reason for this lies, according to Bohr, in the fact that the tension curve is influenced by the form of union of the part of the hæmoglobin containing iron with the iron-free part, and that this union is changed because of changes in the iron-free part, as by the splitting off of lecithin, etc. The tension curve of the oxygen in the blood can, according to Bohr, be determined only by direct experiments on the blood itself and not by experiments upon hæmoglobin solutions.

The elucidation of these conditions is of the very greatest importance, as the dependence of the reaction between OHb, Hb, and O upon the law of mass-action is naturally of the very greatest moment for the taking up of oxygen in the lungs and the giving up of the same to the tissues. The dissociation of the oxyhæmoglobin makes it also possible to completely expel the oxygen from a hæmoglobin solution or from blood by means of a vacuum or by passing an indifferent gas through the blood.

Oxyhæmoglobin, which is generally considered as a weak acid, is dextrorotatory, according to Gamgee.[1] The specific rotation for light of medium wave-lengths of C is $(\alpha)C = $ about $+10°$, which corresponds also for carbon-monoxide hæmoglobin. The hæmoglobin is also, like carbon-monoxide hæmoglobin (COHb) and methæmoglobin (MHb), diamagnetic, while the hæmatin, which is richer in iron, is strongly magnetic (Gamgee[2]). On passing an electric current through an oxyhæmoglobin solution, the pigment first separates unchanged at the anode in a colloidal but still soluble form, and is then gradually transferred to the cathode in the colloidal state (Gamgee[3]). This transportation of the colloidal hæmoglobin may also be made to take place through an animal membrane or through parchment paper. According to Gamgee, the hæmoglobin probably exists in such a colloidal condition in the blood-corpuscles.

Oxyhæmoglobin has been obtained in crystals from several varieties of blood. These crystals are blood-red, transparent, silky, and may be 2–3 mm. long. The oxyhæmoglobin from squirrel's blood crystallizes in six-sided plates of the hexagonal system; the other varieties of blood yield needles, prisms, tetrahedra, or plates which belong to the rhombic system.[1] The quantity of water of crystallization varies between 3–10 per cent for the different oxyhæmoglobins. When completely dried at a low temperature over sulphuric acid the crystals may be heated to 110–115° C. without decomposition. At higher temperatures, somewhat above 160° C., they decompose, giving an odor of burnt horn, and leave, after complete combustion, an ash consisting of oxide of iron. The oxyhæmoglobin crystals from difficultly crystallizable kinds of blood, for example from such as ox's, human, and pig's blood, are easily soluble in water. The oxyhæmoglobins from easily crystallizable blood, as from that of the horse, dog, squirrel, and guinea-pig, are soluble with difficulty in the order above given. The oxyhæmoglobin dissolves more easily in a very dilute solution of alkali carbonate than in pure water, and this solution may be kept. The presence of a little too much alkali causes the oxyhæmoglobin to quickly decompose. The crystals are insoluble without decolorization in absolute alcohol. According to NENCKI,[2] it is hereby converted into an isomeric or polymeric modification, called by him *parahæmoglobin*. Oxyhæmoglobin is insoluble in ether, chloroform, benzene, and carbon disulphide.

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[1] Hofmeister's Beiträge, 4. [2] Proceedings of Roy. Society, 68. [3] *Ibid.*, 70.

A solution of oxyhæmoglobin in water is precipitated by many metallic salts, but is not precipitated by sugar of lead or basic lead acetate. On heating the watery solution it decomposes at about 70° C., and splits off protein and hæmatin. It is also readily decomposed by acids, alkalies, and many metallic salts. It gives the ordinary reactions for proteins with those protein reagents which first decompose the oxyhæmoglobin with the splitting off of protein. Oxyhæmoglobin, like the other blood-pigments, has a direct oxidizing action upon tincture of guaiacum. It has, on the other hand, like all blood-pigments containing iron, the property of an "ozone transmitter" in that it turns tincture of guaiacum blue in the presence of reagents containing peroxide, such as old turpentine.

A sufficiently dilute solution of oxyhæmoglobin or arterial blood shows a spectrum with two absorption-bands between the FRAUNHOFER lines *D* and *E*. The one band, α, which is narrower but darker and sharper, lies on the line *D*; the other, broader, less defined and less dark band, β, lies at *E*. The middle of the first band corresponds to a wave-length $\lambda = 578.1$ and the second $\lambda = 541.7$. These bands can be detected in a layer 1 cm. thick of a 0.1 p. m. solution of oxyhæmoglobin. In a still weaker dilution the band β first disappears. By increased concentration of the solution the two bands become broader, the space between them smaller or entirely obliterated, and at the same time the blue and violet part of the spectrum is darkened. The oxyhæmoglobin may be differentiated from other coloring-matters having a similar absorption-spectrum by its behavior towards reducing substances.[1] (See p. 203.)

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[1] The observation of Uhlik (Pflüger's Arch., 104) that the hæmoglobin from horse-blood can also crystallize in hexagonal six-sided plates seems to be due to the fact that he had hæmoglobin and not oxyhæmoglobin.

[2] Nencki and Sieber, Ber. d. d. chem. Gesellsch., 18. According to Krüger (see Biochem Centralbl., I, 40, 463) hæmoglobin is somewhat changed by alcohol as well as by chloroform.

The observation of PIETTRE and VILA that so-called laky blood and oxyhæmoglobin solutions in thick layers also show a third band in the red ($\lambda=634$) depends in all probability, as also claimed by VILLE and DERRIEN,[2] upon a partial formation of methæmoglobin.

A great many methods have been proposed for the preparation of oxyhæmoglobin crystals, but in their chief features they all agree with the following one suggested by HOPPE-SEYLER: The washed blood-corpuscles (best those from the dog or the horse) are stirred with 2 vols. water and then shaken with ether. After decanting the ether and allowing the ether which is retained by the blood solution to evaporate in an open dish in the air, cool the filtered blood solution to 0° C., add while stirring ¼ vol. of alcohol also cooled, and allow to stand a few days at $-5°$ to $-10°$ C. The crystals which separate may be repeatedly recrystallized by dissolving in water of about 35° C., cooling, and adding cooled alcohol as above. Lastly, they are washed with cooled water containing alcohol (¼ vol. alcohol) and dried in vacuum at 0° C. or a lower temperature.[3]

For the preparation of oxyhæmoglobin crystals in small quantities from easily crystallizable blood, it is often sufficient to stir a drop of blood with a little water on a microscope slide and allow the mixture to evaporate so that the drop is surrounded by a dried ring. After covering with a cover-glass, the crystals gradually appear radiating from the ring. These crystals are formed more surely if the blood is first mixed with some water in a test-tube and shaken with ether and a drop of the lower deep-colored liquid treated as above on the slide.

Hæmoglobin, also called REDUCED HÆMOGLOBIN OF PURPLE CRUORIN (STOKES[4]), occurs only in very small quantities in arterial blood, in larger quantities in venous blood, and is nearly the only blood-coloring matter after asphyxiation.

Hæmoglobin is much more soluble than the oxyhæmoglobin, and it can therefore be obtained as crystals only with difficulty. These crystals are as a rule isomorphous with the corresponding oxyhæmoglobin crystals, but are darker, having a shade towards blue or purple, and are decidedly more pleochromatic. The hæmoglobin from horse-blood has also been obtained by UHLIK[1] in hexagonal six-sided plates. Its solutions in water are darker and more violet or purplish than solutions of oxyhæmoglobin of the same concentration. They absorb the blue and the violet rays of the spectrum in a less marked degree, but strongly absorb the rays lying between *C* and *D*. In proper dilution the solution shows a spectrum with one broad, not sharply defined band between *D* and *E*, whose darkest part corresponds to the wave-length $\lambda = 555$. This band does not lie in the middle between *D* and *E*, but is towards the red end of the spectrum, a little over the line *D*. A hæmoglobin solution actively absorbs oxygen from the air and is converted into an oxyhæmoglobin solution.

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[1] Zeitschr. f. Biologie, **34**, contains the investigations of GAMGEE on the absorption of the ultra-violet rays by the blood pigment. It also contains some of the earlier investigations.

[2] Piettre and Vila, Compt. rend., **140**; Ville and Derrien, *ibid.*, **140**.

[3] In regard to the preparation of oxyhæmoglobin, see also Hoppe-Seyler-Thierfelder's Handbuch, 7. Aufl.; also the works cited in foot-note 1, p. 198; also Schuurmanns-Stekhoven, Zeitschr. f. physiol. Chem., **33**, 296; see also Bohr, Skand. Arch. f. Physiol., **3**.

[4] Philosophical Magazine, 28, No. 190, Nov., 1864.

A solution of oxyhæmoglobin may be easily converted into a solution having the spectrum of hæmoglobin by means of a vacuum, by passing an indifferent gas through it, or by the addition of a reducing substance, as, for example, an ammoniacal ferrous-tartrate solution (STOKES' reduction liquid). If an oxyhæmoglobin solution or arterial blood is kept in a sealed tube, we observe a gradual consumption of oxygen and a reduction of the oxyhæmoglobin into hæmoglobin. If the solution has a proper concentration, a crystallization of hæmoglobin may occur in the tube at lower temperatures (HÜFNER[2]).

Pseudohæmoglobin. LUDWIG and SIEGFRIED[3] have observed that blood which has been reduced by hyposulphites so completely that the oxyhæmoglobin spectrum disappears and only the hæmoglobin spectrum is seen, yields large amounts of oxygen when exposed to a vacuum. Blood which has been reduced by the passage of a stream of hydrogen through it until the oxyhæmoglobin spectrum disappears acts in the same manner. Hence a loose combination of hæmoglobin and oxygen exists which gives the hæmoglobin spectrum, and this combination is called pseudohæmoglobin by LUDWIG and SIEGFRIED. Pseudohæmoglobin, whose presence has been detected in asphyxiation blood from dogs, is considered by HAMMARSTEN as an intermediate step between hæmoglobin and oxyhæmoglobin on the reduction of the latter. The occurrence of pseudohæmoglobin does not seem to have been positively proved.[4]

Methæmoglobin. This name has been given to a coloring-matter which is easily obtained from oxyhæmoglobin as a transformation product and which has been correspondingly found in transudates and cystic fluids containing blood, in urine in hæmaturia or hæmoglobinuria, also in urine and blood on poisoning with potassium chlorate, amyl nitrite or alkali nitrite, and many other bodies.

Methæmoglobin does not contain any oxygen in molecular or dissociable combination, but still the oxygen seems to be of importance in the formation of methæmoglobin, because it is formed from oxyhæmoglobin and not from hæmoglobin in the absence of oxygen or oxidizing agents. If arterial blood be sealed up in a tube, it gradually consumes its oxygen and becomes venous, and by this absorption of oxygen a little methæmoglobin is formed. The same occurs on the addition of a small quantity of acid to the blood. By the spontaneous decomposition of blood some methæmoglobin is formed, and by the action of ozone, potassium permanganate, potassium ferricyanide, chlorates, nitrites, nitrobenzene, pyrogallol, pyrocatechin, acetanilide, and certain other bodies on the blood an abundant formation of methæmoglobin takes place.

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[1] Pflüger's Arch., 104.

[2] Zeitschr. f. physiol. Chem., 4; see also Uhlik, l. c.

[3] Arch. f. (Anat. u.) Physiol., 1890; see also Ivo Novi, Pflüger's Archiv, 56.

[4] See Hüfner, Arch. f. (Anat. u.) Physiol., 1894, 140.

According to the investigations of Hüfner, Külz, and Otto[1] methæmoglobin contains just as much oxygen as oxyhæmoglobin, but it is more strongly combined. By the action of potassium ferricyanide or potassium permanganate upon oxyhæmoglobin first 1 molecule oxygen (i.e., the entire quantity of loosely combined oxygen) is split off and in the subsequent methæmoglobin formation either two oxygen atoms (Haldane) or two hydroxyl groups are combined (Hüfner, v. Zeynek[2]). Methæmoglobin solutions are reduced to hæmoglobin by reducing agents. Jäderholm and Saarbach claim that methæmoglobin is first converted into oxyhæmoglobin and then into hæmoglobin by reducing substances, while others (Hoppe-Seyler and Araki[3]) dispute this.

According to Hüfner and Reinbold[4] 1 gram methæmoglobin can take up 2.685 c.c. nitric oxide.

Methæmoglobin crystallizes, as first shown by Hüfner and Otto, in brownish-red needles, prisms, or six-sided plates. It dissolves easily in water; the solution has a brown color and becomes a beautiful red on the addition of alkali. The solution of the pure substance is not precipitated by basic lead acetate alone, but by basic lead acetate and ammonia. The absorption-spectrum of a watery or acidified solution of methæmoglobin is, according to Jäderholm and Bertin-Sans, very similar to that of hæmatin in acid solution, but is easily distinguished from the latter since, on the addition of a little alkali and a reducing substance, the former passes over to the spectrum of reduced hæmoglobin, while a hæmatin solution under the same conditions gives the spectrum of an alkaline hæmochromogen solution (see below). Methæmoglobin in alkaline solution shows two absorption-bands which are like the two oxyhæmoglobin bands, but they differ from these in that the band β is stronger than α. By the side of the band α and united with it by a shadow lies a third fainter band between C and D, near to D. According to other investigators, Araki and Dit-

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[1] See Otto, Zeitschr. f. physiol. Chem., **7**.

[2] Haldane, Journ. of Physiol., **22**; v. Zeynek, Arch. f. (Anat. u.) Physiol., 1899; Hüfner, *ibid.*

[3] Jäderholm, Zeitschr. f. Biologie, **16**; Saarbach, Pflüger's Arch., **28**; Araki, Zeit-ır. f. physiol. Chem., **14**.

[4] Arch. f. (Anat. u.) Physiol., 1904, Suppl.

TRICH, a neutral or faintly acid methæmoglobin solution shows only one characteristic band, α, between *C* and *D*, whose middle corresponds to about $\lambda=634$. The two bands between *D* and *E* are only due to contamination with oxyhæmoglobin (MENZIES[1]).

The statements as to the action of sodium fluoride upon hæmoglobin and methæmoglobin are somewhat contradictory.[2]

Crystallized methæmoglobin may be easily obtained by treating a concentrated solution of oxyhæmoglobin with a sufficient quantity of concentrated potassium-ferricyanide solution to give the mixture a porter-brown color. After cooling to 0° C. add ¼ vol. cooled alcohol and allow the mixture to stand a few days in the cold. The crystals may be easily purified by recrystallizing from water by the addition of alcohol.

Cyanmethæmoglobin (cyanhæmoglobin) is, according to HALDANE, identical with photomethæmoglobin (BOCK), which is produced by the influence of sunlight upon a methæmoglobin solution containing potassium ferricyanide. It was first carefully described by R. KOBERT and obtained in a crystalline form by v. ZEYNEK.[3] It is immediately formed in the cold by the action of a hydrocyanic-acid solution upon methæmoglobin, but is formed by its action upon oxyhæmoglobin only at the body temperature. The neutral or faintly alkaline solutions show a spectrum which is very similar to the hæmoglobin spectrum.

Acid hæmoglobin is a coloring-matter produced by the action of very weak acids upon oxyhæmoglobin, which according to HARNACK[4] is not, as used to be admitted, identical with methæmoglobin.

Carbon-monoxide Hæmoglobin[5] is the molecular combination between 1 molecule of hæmoglobin and 1 molecule of CO, according to HÜFNER,[6] which contains 1.34 c.c. of carbon monoxide (at 0° and 760 mm. Hg) for 1 gram hæmoglobin. This combination is stronger than the oxygen combination of hæmoglobin. The oxygen is for this reason easily driven out of oxyhæmoglobin by carbon monoxide, and this explains the poisonous action of this gas, which kills by the expulsion of the oxygen of the blood. In regard to the division of the blood-pigments between the carbon monoxide and oxygen under different partial pressures of both gases in the air, we must refer to the investigations of HÜFNER,[1] whose results are tabulated.

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[1] Jäderholm, l. c.; Bertin-Sans, Comp. rend., **106**; Dittrich, Arch. f. exp. Path. u. Pharm., **29**; Menzies, Journ. of Physiol., **17**. Important references on methæmoglobin are given by Otto, Pflüger's Arch., **31**.

[2] Piettre and Vila, Compt. rend., **140**; Ville and Derrien, *ibid.*, **140**.

[3] Haldane, Journ. of Physiol., **25**; Bock, Skand. Arch. f. Physiol., **6**; Kobert, Pflüger's Arch., **82**; v. Zeynek, Zeitschr. f. physiol. Chem., **33**.

[4] Zeitschr. f. physiol. Chem., **26**.

[5] In reference to carbon-monoxide hæmoglobin, see especially Hoppe-Seyler, Med.-chem. Untersuch., 201; Centralbl. f. d. med. Wissensch., 1864 and 1865; Zeitschr. f. physiol. Chem., **1** and **13**.

[6] Arch. f. (Anat. u.) Physiol., 1894. On the dissociation constant of carbon-monoxide hæmoglobin, see *ibid.*, 1895. In regard to the contradictory statements of Saint-Martin and others and their disproval, see Hüfner, Arch. f. (Anat. u.) Physiol., 1903.

The carbon monoxide can be driven out by a vacuum as well as by passing an indifferent gas or oxygen or nitric oxide through the solution for a long time, and in these cases hæmoglobin, oxyhæmoglobin, or nitric-oxide hæmoglobin are formed. The carbon monoxide is also expelled by potassium ferricyanide and methæmoglobin is formed (HALDANE[2]).

Carbon-monoxide hæmoglobin is formed by saturating blood or a hæmoglobin solution with carbon monoxide, and may be obtained as crystals by the same means as oxyhæmoglobin. These crystals are isomorphous with the oxyhæmoglobin crystals, but are less soluble and more stable, and their bluish-red color is more marked. For the detection of carbon-monoxide hæmoglobin, its absorption-spectrum is of the greatest importance. This spectrum shows two bands which are very similar to those of oxyhæmoglobin, but they occur more towards the violet part of the spectrum. The middle of the first band corresponds to $\lambda=572$ and the second to $\lambda=536$. These bands do not change noticeably on the addition of reducing substances; this constitutes an important difference between carbon-monoxide hæmoglobin and oxyhæmoglobin. If the blood contains oxyhæmoglobin and carbon-monoxide hæmoglobin at the same time, we obtain on the addition of a reducing substance (ammoniacal ferro-tartrate solution) a mixed spectrum originating from the hæmoglobin and carbon-monoxide hæmoglobin.

A great many reactions have been suggested for the detection of carbon-monoxide hæmoglobin in medico-legal cases. A simple and at the same time a good one is HOPPE-SEYLER'S alkali test. The blood is treated with double its volume of caustic-soda solution of 1.3 sp. gr., by which ordinary blood is converted into a dingy brownish mass, which when spread out on porcelain is brown with a shade of green. Carbon-monoxide blood gives under the same conditions a red mass, which if spread out on porcelain shows a beautiful red color. Several modifications of this test have been proposed. Another very good reagent is tannic acid, which gives with dilute normal blood a brownish-green precipitate and with carbon-monoxide blood a pale crimson-red precipitate.[3]

As according to BOHR there are several oxyhæmoglobins, so also, according to BOHR and BOCK,[4] there are several carbon-monoxide hæmoglobins, with different amounts of carbon monoxide. As hæmoglobin can unite with oxygen and carbon dioxide simultaneously, as shown by BOHR and TORUP, so also can it unite with carbon monoxide and carbon dioxide simultaneously and independently of each other.

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[1] Arch. f. exp. Path. u. Pharm., 48.

[2] Journ. of Physiol., 22.

[3] In regard to this test (as suggested by Kunkel) and others we refer to Kostin, Pflüger's Arch., 84, which contains a very excellent summary of the literature on the subject.

[4] Centralbl. f. Physiol., 8, and Maly's Jahresber., 25.

Carbon-monoxide methæmoglobin has been prepared by WEIL and v. ANREP by the action of potassium permanganate on carbon-monoxide hæmoglobin, but this is contradicted by BERTIN-SANS and MOITESSIER.[1] **Sulphur methæmoglobin** is the name given by HOPPE-SEYLER to that coloring-matter which is formed by the action of sulphuretted hydrogen upon oxyhæmoglobin. The solution has a greenish-red, dirty color, and shows two absorption-bands between C and D. This coloring-matter is claimed to be the greenish color seen on the surface of putrefying flesh. According to HARNACK [2] the conditions are different when H_2S is passed through an oxygen-free solution of hæmoglobin (or carbon-monoxide hæmoglobin). The sulphhæmoglobin thus formed shows one band in the red between C and D.

Carbon-dioxide Hæmoglobin, *Carbohæmoglobin.* Hæmoglobin, according to BOHR and TORUP,[3] also forms a molecular combination with carbon dioxide whose spectrum is similar to that of hæmoglobin. According to BOHR there are three different carbohæmoglobins, namely, α-, β-, and γ-carbohæmoglobin, in which 1 gram combines with respectively 1.5, 3, and 6 c.c. CO_2 (measured at 0° C. and 760 mm.) at 18° C. and a pressure of 60 mm. mercury. If a hæmoglobin solution is shaken with a mixture of oxygen and carbon dioxide, the hæmoglobin combines loosely with the oxygen as well as with the carbon dioxide, independently of each other, just as if each gas existed alone (BOHR). He considers that the two gases are combined with different parts of the hæmoglobin, that is, the oxygen with the pigment nucleus and the carbon dioxide with the protein component. BOHR has given an equilibrium formula for the carbon-dioxide absorption of hæmoglobin at different carbon-dioxide tensions, and the results obtained on calculation, using this formula, correspond very well with the results obtained directly. Attention must be called to the fact that, as observed by TORUP, hæmoglobin is in part readily decomposed by the carbon dioxide with the splitting off of some protein.

Nitric-oxide Hæmoglobin is also a crystalline molecular combination which is even stronger than the carbon-monoxide hæmoglobin. Its solution shows two absorption-bands which are paler and less sharp than the carbon-monoxide hæmoglobin bands, and they do not disappear on the addition of reducing bodies. Hæmoglobin also forms a molecular combination with acetylene.

Hæmorrhodin is the name given by LEHMANN to a beautiful red pigment soluble in alcohol and ether, which is extracted from meat and meat products by boiling alcohol and which seems to be produced by the action of small amounts of nitrites. Another pigment isolated by LEWIN[1] from the blood of animals poisoned by phenylhydrazine has been called *hæmoverdin*. By heating a solution of blood-pigment treated with caustic potash and mixed with alcohol to 60° C. we obtain, according to v. KLAVEREN, a pigment which he calls *kathæmoglobin*, but called by ARNOLD,[2] who first obtained it, *neutral hæmatin*, which is produced by the splitting off of a ferruginous complex. This pigment still contains protein, but is poorer in iron than the hæmoglobin or methæmoglobin and probably forms an intermediary product in the conversion of the above into hæmatin.

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[1] v. Anrep, Arch. f. (Anat. u.) physiol., 1880; Sans and Moitessier, Compt. rend., **113**.

[2] Med.-chem. Untersuch., 151. See Araki, Zeitschr. f. physiol. Chem., **14**; Harnack, l. c.

[3] Bohr, Extrait du Bull. de l'Acad. Danoise, 1890; Centralbl. f. Physiol., **4** and **17**; Torup, Maly's Jahresber., **17**.

Decomposition products of the blood-pigments. By its decomposition hæmoglobin yields, as previously stated, a *protein*, which has been called *globin* (PREYER, SCHULZ), and a ferruginous *pigment* as chief products. According to LAWROW 94.09 per cent protein, 4.47 per cent hæmatin, and 1.44 per cent other bodies are produced in this decomposition. The globin, which was isolated and studied by SCHULZ,[3] differs from most other proteins by containing a high amount of carbon, 54.97 per cent, with 1698. per cent of nitrogen. It is insoluble in water, but very easily soluble in acids or alkalies. It is not dissolved by ammonia in the presence of ammonium chloride. Nitric acid precipitates it in the cold but not when warm. It may be coagulated by heat, but the coagulum is readily soluble in acids. Because of these reactions it is considered as a histone by SCHULZ.

On hydrolytic cleavage globin (from horse-blood) yields, according to ABDERHALDEN,[4] the ordinary cleavage products of the proteins and especially leucine, 29 per cent. It is also important to call attention to the large amount of histidine, 10.96 per cent, while the quantities of arginine and lysine were only 5.42 and 4.28 per cent respectively.

The pigment split off is different, depending upon the conditions under which the cleavage takes place. If the decomposition takes place in the absence of oxygen, a coloring-matter is obtained which is called by HOPPE-SEYLER *hæmochromogen*, by other investigators (STOKES) *reduced hæmatin*. In the presence of oxygen, hæmochromogen is quickly oxidized to hæmatin, and there is therefore obtained in this case *hæmatin* as a colored decomposition product. As hæmochromogen is easily converted by oxygen into hæmatin, so this latter may be reconverted into hæmochromogen by reducing substances.

Hæmochromogen was discovered by HOPPE-SEYLER.[5] It is, according to HOPPE-SEYLER, the colored atomic group of hæmoglobin and of its combinations with gases, and this atomic group is combined with proteins in the pigment. The characteristic absorption of light depends on the hæmochromogen, and it is also this atomic group which binds in the oxyhæmoglobin 1 molecule of oxygen and in the carbon-monoxide hæmoglobin 1 molecule of carbon monoxide with 1 atom of iron. Hæmochromogen is produced in an alkaline solution of hæmatin by the action of reducing bodies. By the reduction of hæmatin in alcoholic ammoniacal solution by means of hydrazine v. ZEYNEK[1] was able to obtain the solid brownish-red ammonia combination.

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[1] K. B. Lehmann, Sitzungsber. d. phys.-med. Gesellsch. Würzburg, 1899; Lewin, Compt. rend., **133**.

[2] v. Klaveren, Zeitschr. f. physiol. Chem., **33**; Arnold, *ibid.*, **29**.

[3] Lawrow, *ibid.*, **26**; Schulz, *ibid.*, **24**; Preyer, Die Blutkristalle, Jena, 1871.

[4] Zeitschr. f. physiol. Chem., **37**.

[5] *Ibid.*, **13**.

Hæmochromogen combines, as HOPPE-SEYLER first showed, also with carbon monoxide. This compound, which in aqueous solution gives a spectrum similar to oxyhæmoglobin, has been obtained by PREGL[2] in the solid condition as a deep-violet powder which is insoluble in absolute alcohol. In opposition to hæmoglobin the hæmochromogen combines with oxygen more firmly than with carbon monoxide. The assumption of HOPPE-SEYLER that this compound is a combination of 1 molecule hæmochromogen and therefore contains 1 molecule carbon monoxide for 1 molecule of iron has been experimentally substantiated by HÜFNER and KÜSTER and by PREGL.[3]

An alkaline hæmochromogen solution has a beautiful cherry-red color. It shows two absorption-bands, first described by STOKES, one of which is dark and whose center corresponds to $\lambda=556.4$ between D and E, and a second broader band, less dark, which covers the FRAUNHOFER lines E and b. The middle of this band corresponds to $\lambda=520.4$. In acid solution hæmochromogen shows four bands, which, according to JÄDERHOLM,[4] depend on a mixture of hæmochromogen and hæmatoporphyrin (see below), this last formed by a partial decomposition resulting from the action of the acid.

MILROY[5] from an alcoholic solution of hæmatin containing oxalic acid, after driving out the air by means of hydrogen gas, gradually obtained an acid solution of reduced hæmatin (hæmochromogen) by means of zinc dust. This solution showed one absorption-band between D and E.

Hæmochromogen may be obtained as crystals by the action of caustic soda on hæmoglobin at 100° C. in the absence of oxygen (HOPPE-SEYLER). By the decomposition of hæmoglobin by acids (of course in the absence of air) we obtain hæmochromogen contaminated with a little hæmatoporphyrin. An alkaline hæmochromogen solution is easily obtained by the action of a reducing substance (STOKES' reduction liquid) on an alkaline hæmatin solution. An ammoniacal solution of hæmatin on reduction with hydrazine yields hæmochromogen very easily. An alcoholic, alkaline hydrazine solution is also recommended by RIEGLER[1] as a reagent for blood-pigments, converting them into hæmochromogen.

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[1] Zeitschr. f. physiol., Chem., 25.

[2] *Ibid.*, 44.

[3] Hüfner and Küster, Arch. f. (Anat. u.) Physiol., 1904, Suppl.; Pregl, l. c.

[4] Nord. Med. Arkiv., 16.

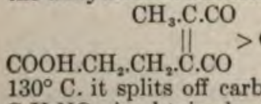
[5] Journ. of Physiol., 32.

Hæmatin, also called OXYHÆMATIN, is sometimes found in old transudates. It is formed by the action of the gastric or pancreatic juices on oxyhæmoglobin, and is therefore also found in the fæces after hemorrhage in the intestinal canal, and also after a meat diet and food rich in blood. It is stated that hæmatin may occur in urine after poisoning with arseniuretted hydrogen. As shown above, the hæmatin is formed by the decomposition of oxyhæmoglobin, or at least of hæmoglobin, in the presence of oxygen.

The statements in regard to the composition of hæmatin are rather contradictory, which seems to be due to the fact that the substance, hæmin (see below), from which the formula of hæmatin is derived, has a somewhat different composition, dependent upon various conditions. According to HOPPE-SEYLER hæmatin has the formula $C_{34}H_{34}N_4FeO_5$, and from the recent investigations upon hæmin, which will be mentioned below, this formula seems to be now generally accepted. According to this formula 1 atom of iron occurs with every 4 atoms of nitrogen. According to CLOETTA, and also ROSENFELD,[2] hæmatin has the formula $C_{30}H_{34}N_3FeO_3$, with 1 atom of iron for every 3 atoms of nitrogen.

Hæmatin is very resistant towards boiling concentrated caustic potash as well as towards boiling hydrochloric acid. It dissolves in concentrated sulphuric acid and is converted into hæmatoporphyrin with the splitting off of iron. On heating dry hæmatin it yields abundant pyrrol. On reduction with tin and hydrochloric acid a body similar to urobilin is formed. As an oxidation product of hæmatin in glacial acetic acid with potassium bichromate or chromium trioxide, KÜSTER[3] obtained the imide of the tribasic hæmatinic acid, $C_8H_9NO_4$, which is also produced on the oxidation of hæmatoporphyrin and bilirubin.

The imide of the tribasic hæmatinic acid, which is a derivative of maleïc acid and probably has the formula $C_5H_7(COOH)<\begin{smallmatrix}CO\\CO\end{smallmatrix}>NH$, is readily transformed into the anhydride of the tribasic hæmatinic acid, $C_8H_8O_5$, having the probable formula



$$\begin{array}{l}\quad\quad CH_3.C.CO\\ \quad\quad\quad\quad\| \quad\quad >O.\\ COOH.CH_2.CH_2.C.CO\end{array}$$

On heating the imide with alcoholic ammonia to 130° C. it splits off carbon dioxide, and the imide of the bibasic hæmatinic acid, $C_7H_9NO_2$, is obtained. From this imide on saponification with baryta-water we obtain the barium salt of an acid whose anhydride is methyl-ethyl maleïc-acid anhydride,

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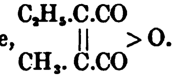
[1] Zeitschr. f. analyt. Chem., **43**.

[2] Hoppe-Seyler, Med.-chem. Untersuch., p. 525; Cloetta, Arch. f. exp. Path. u. Pharm., **36**; Rosenfeld, *ibid.*, **40**.

[3] Beiträge zur Kenntnis des Hämatins, Tübingen, 1896; Ber. d. d. chem. Gesellsch., **27**, **30**, **32**, and **35**; Annal. d. Chem. u. Pharm., **315**, and Zeitschr. f. physiol. Chem., **28**, **40**, and **44**.



$$\begin{matrix} C_2H_5.C.CO \\ \| \\ CH_3.C.CO \end{matrix} > O.$$

The yield of hæmatinic acids is so great that KÜSTER considers that at least three if not four molecules $C_8H_9NO_4$ are formed from one hæmatin molecule. On heating hæmatinic acid ester with alcoholic ammonia in a tube to 130° KÜSTER obtained a colored product whose bluish-violet aqueous solution gave a spectrum with two bands which in position were similar to the oxyhæmoglobin spectrum.

Hæmatin is amorphous, dark brown or bluish black. It may be heated to 180° C. without decomposition; on burning it leaves a residue consisting of iron oxide. It is insoluble in water, dilute acids, alcohol, ether, and chloroform, but it dissolves slightly in warm glacial acetic acid. Hæmatin dissolves in acidified alcohol or ether. It easily dissolves in alkalies, even when very dilute. The alkaline solutions are dichroitic; in thick layers they appear red by transmitted light and in thin layers greenish. The alkaline solutions are precipitated by lime- and baryta-water, as also by solutions of neutral salts of the alkaline earths. The acid solutions are always brown.

An acid hæmatin solution absorbs the red part of the spectrum only slightly and the violet parts strongly. The solution shows a rather sharply defined band between *C* and *D*, whose position may change with the variety of acid used as a solvent. Between *D* and *F* a second, much broader, less sharply defined band occurs, which by proper dilution of the liquid is converted into two bands. The one between *b* and *F*, lying near *F*, is darker and broader; the other, between *D* and *E*, lying near *E*, is lighter and narrower. Also by proper dilution a fourth very faint band is observed between *D* and *E*, lying near *D*. Hæmatin may thus in acid solution show four absorption-bands; ordinarily one sees distinctly only the bands between *C* and *D* and the broad, dark band—or the two bands—between *D* and *F*. In alkaline solution hæmatin shows a broad absorption-band, which lies in greatest part between *C* and *D*, but reaches a little over the line *D* towards the right in the space between *D* and *E*. As the position of the hæmatin bands in the spectrum is quite variable, the exact wave-lengths corresponding thereto cannot be given exactly.

Hæmin, HÆMIN CRYSTALS, or TEICHMANN'S CRYSTALS. Hæmin is the hydrochloric-acid ester of hæmatin and is the starting-point in the preparation of the latter.

The statements as to the composition of hæmin are just as variable as those for hæmatin, which is partly due to the fact, as shown by NENCKI and ZALESKI, that the hæmatin, which contains two hydroxyls in the molecule, may form ethers with acids and alkyl radicals, which also yield addition products with indifferent compounds. Thus the hæmin prepared according to NENCKI and SIEBER'S method contains amyl alcohol. SCHALFEJEFF'S hæmin, having the formula $C_{34}H_{33}N_4FeO_4Cl$, is supposed to contain an acetyl group and hence is called acethæmin. Mörner's hæmin, $C_{35}H_{35}N_4FeO_4Cl$, is considered as a monoethyl ether of acethæmin. The investigations of Zaleski, Hetper and Marchlewski, K. Mörner, and especially those of Küster have given explanations of these conditions. The so-called acethæmin does not contain any acetic-acid radical, hence its name is incorrect. Küster, by a new method of purification and recrystallization, has shown that the older various kinds of hæmins were not chemical individuals and that we have only one hæmin. This view is now accepted by Mörner and most of the other investigators, and the formula $C_{34}H_{33}O_4N_4FeCl$ is now given to hæmin. Piettre and Vila [1] dispute this formula, and they claim to have prepared chlorine-free hæmin from pure crystalline oxyhæmoglobin.

Hæmin crystals form in large masses a bluish-black powder, but are so small that they can only be seen by aid of the microscope. They consist of dark-brown or nearly brownish-black long, rhombic, or spool-like crystals, isolated or grouped as crosses, rosettes, or stellar forms. Cubical crystals may also occur, according to Cloetta. They are insoluble in water, dilute acids at the normal temperature, alcohol, ether, and chloroform. They are slightly soluble in glacial acetic acid with heat. They dissolve in acidified alcohol, as also in dilute caustic alkalies or carbonates; and in the last case they form, besides alkali chlorides, soluble hæmatin alkali, from which the hæmatin may be precipitated by an acid.

On shaking with cold aniline and treating first with acetic acid and then with ether, Küster obtained a product, dehydrochloride hæmin, which was poor in the elements of hydrochloric acid and which again took up HCl and was converted into hæmin. By the action of boiling aniline, hydrogen is driven out and a combination with aniline, without loss of iron, takes place.

The principle of the preparation of hæmin crystals in large quantities is as follows: The washed sediment from the blood-corpuscles is coagulated with alcohol or by boiling after dilution with water and the careful addition of acid. The strongly pressed but not dry mass is rubbed with 90–95 per cent alcohol which has been previously treated with oxalic acid or ½–1 per cent concentrated sulphuric acid, and this is allowed to stand several hours at the temperature of the room. The filtrate is warmed to about 70° C., treated with hydrochloric acid (for each litre of filtrate add 10 c.c. 25 per cent hydrochloric acid diluted with alcohol—Mörner), and allowed to stand in the cold. The crystals, which separate in one or two days, are first washed with alcohol and then with water. For particulars as to the various methods of preparation and purification we refer the reader to the above-cited works of NENCKI and SIEBER, CLOETTA, MÖRNER, ROSENFELD, NENCKI and ZALESKI (SCHALFEJEFF), and especially to KÜSTER.[1]

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[1] Nencki and Zaleski, Zeitschr. f. physiol. Chem., **30**; Nencki and Sieber, Arch. f. exp. Path. u. Pharm., **18** and **20**, and Ber. d. d. chem. Gesellsch., **18**; Schalfejeff with Nencki and Zaleski, l. c.; Bialobrzeski, Arch. des scienc. biol. de St. Pétersbourg, **5**; K. Mörner, Nord. Med. Arkiv, Festband, 1897, Nos. 1 and 26, and Zeitschr. f. physiol. Chem., **41**; Zaleski, *ibid.*, **37**; Hetper and Marchlewski, *ibid.*, **41** and **42**; Küster, *ibid.*, **40**; Piettre and Vila, Compt. rend., **141**, p. 734.

Hæmatin is obtained on dissolving the hæmin crystals in very dilute caustic alkali and precipitating with an acid.

In preparing hæmin crystals in small quantities proceed in the following manner: The blood is dried after the addition of a small quantity of common salt, or the dried blood may be rubbed with a trace of the same. The dry powder is placed on a microscope slide, moistened with glacial acetic acid, and then covered with the cover-glass. Add, by means of a glass rod, more glacial acetic acid by applying the drop at the edge of the cover-glass until the space between the slide and the cover-glass is full. Now warm over a very small flame, with the precaution that the acetic acid does not boil and pass with the powder from under the cover-glass. If no crystals appear after the first warming and cooling, warm again, and if necessary add some more acetic acid. After cooling, if the experiment has been properly performed, a number of dark-brown or nearly black hæmin crystals of varying forms will be seen.

In regard to the preparation of iodohæmatin and the use of the same for the detection of blood we must refer to STRZYZOWSKI's communication.[2]

By the action of acids upon hæmochromogen, hæmatin, or hæmin a new iron-free pigment, which was first closely studied by HOPPE-SEYLER and called *hæmatoporphyrin*, is produced. According to the method of preparation hæmatoporphyrins having different solubilities and whose relationship to each other is not perfectly clear are produced, but all show the same characteristic absorption-spectrum. The best-studied hæmatoporphyrin is the one obtained according to NENCKI and SIEBER'S method, by the action of glacial acetic acid saturated with hydrobromic acid upon hæmin crystals, best at the temperature of the body (NENCKI and ZALESKI[3]).

Hæmatoporphyrin, $C_{16}H_{18}N_2O_3$, or $C_{34}H_{38}N_4O_6$ according to ZALESKI.[4] This pigment, according to MACMUNN,[5] occurs as a physiological pigment in certain animals. It occurs, as shown by GARROD and SAILLET, as a normal constituent, although only as traces, of human urine. It occurs in greater quantities in human urine after the use of sulphonal (see Chapter XV).

The formation of hæmatoporphyrin from hæmatin can be expressed by the following equation if we start with the above formula for hæmin and ZALESKI's formula for hæmatoporphyrin:

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[1] Zeitschr. f. physiol. Chem., 40.

[2] Therapeut. Monatshefte, 1901 and 1902.

[3] Hoppe-Seyler, Med.-chem. Untersuch., 528; Nencki and Sieber, Monatshefte f. Chem., 9, and Arch. f. exp. Path. u. Pharm., 18, 20, and 24; Nencki and Zaleski, Zeitschr. f. physiol. Chem., 30.

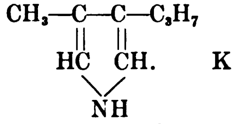
[4] Zeitschr. f. physiol. Chem., 37, 54.

[5] Journ. of Physiol., 7.

$$C_{34}H_{33}N_4O_4FeCl + 2HBr + 2H_2O = C_{34}H_{38}N_4O_6 + FeBr_2 + HCl.$$

On heating hæmatoporphyrin it generates an odor of pyrrol. On oxidation with bichromate and glacial acetic acid it yields hæmatinic acid (see page 210). A pigment closely allied to the urinary pigment urobilin has been obtained by the action of reducing substances on hæmatoporphyrin (HOPPE-SEYLER, NENCKI and SIEBER, LE NOBEL, MACMUNN). On the administration of hæmatoporpyhrin to rabbits, NENCKI and ROTSCHY[1] observed that a part was reduced to a substance similar to urobilin.

Of especial interest are the recent investigations of NENCKI, MARCHLEWSKI, and ZALESKI[2] upon the reduction products of hæmatoporphyrin and their relationship to the chlorophyll derivatives. By the action of glacial acetic acid containing HI and of iodophosphonium upon hæmin or hæmochromogen NENCKI and ZALESKI obtained a markedly characteristic pigment, *mesoporphyrin*, having the formula $C_{16}H_{18}N_2O_2$, or, according to ZALESKI,[3] $C_{34}H_{38}N_4O_4$, and which stands in a certain measure between hæmatoporphyrin, $C_{16}H_{18}N_2O_3$, and the chlorophyll derivative *phylloporphyrin*, $C_{16}H_{18}N_2O$, which is very similar to hæmatoporphyrin. By the action of the same reducing agent upon hæmin or hæmochromogen, but under other conditions, we obtain *hæmopyrrol*, $C_8H_{13}N$, a colorless oil, which in the air gradually changes into urobilin. Hæmopyrrol is produced by the action of the same reducing agents upon the chlorophyll derivative *phyllocyanin* (NENCKI and MARCHLEWSKI), which, as above remarked, shows a close relationship between the blood-pigment and chlorophyll.

According to NENCKI and ZALESKI hæmopyrrol is probably 3-methyl-4-*n*-propylpyrrol,  KÜSTER obtained an imide from hæmopyrrol on oxidation which was probably a derivative of methylpropylmaleïc acid. As hæmatinic acid is undoubtedly a maleïc-acid derivative, it was of interest to prove the correctness of the above formula of hæmopyrrol, and with this purpose in view KÜSTER and HAAS[4] have compared the synthetically prepared imide of methylpropylmaleïc acid with the imide obtained from hæmopyrrol. The two bodies were not identical, therefore the above constitutional formula is questioned. The attempt of BURACZEWSKI and MARCHLEWSKI [1] to prepare hæmopyrrol artificially from methylpropylmaleic-acid imide yielded a product similar to hæmopyrrol, which on oxidation in the air did not yield a typical urobilin but at least a substance closely related thereto. The assumption that hæmopyrrol is a pyrrol derivative is best borne out by the property which hæmopyrrol has of reacting with diazonium compounds with the formation of azo pigments (GOLDMANN, MARCHLEWSKI, HETPER [2]).

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[1] Hoppe-Seyler, l. c., 523; Le Nobel, Pflüger's Arch., **40**; MacMunn, Proc. Roy. Soc., **30**, and Journ. of Physiol., **10**; Nencki and Rotschy, Monatshefte f. Chem., **10**.

[2] See foot-note 1, p. 197.

[3] Zeitschr. f. physiol. Chem., **37**.

[4] Ber. d. d. chem. Gesellsch., **37**.

Hæmatoporphyrin is, according to NENCKI and SIEBER, isomeric with the bile-pigment bilirubin, and like this latter gives a play of colors—green, blue, and yellow—when treated with fuming nitric acid. The hydrochloric-acid compound crystallizes in long brownish-red needles. If the solution in hydrochloric acid is nearly neutralized with caustic soda and then treated with sodium acetate, the pigment separates out as amorphous, brown flakes not readily soluble in amyl alcohol, ether, and chloroform, but readily soluble in ethyl alcohol, alkalies, and dilute mineral acids. The compound with sodium crystallizes as small tufts of brown crystals. The acid alcoholic solutions have a beautiful purple color, which becomes violet-blue on the addition of large quantities of acid. The alkaline solution has a beautiful red color, especially when not too much alkali is present.

An alcoholic solution of hæmatoporphyrin, acidulated with hydrochloric or sulphuric acid, shows two absorption-bands, one of which is fainter and narrower and lies between *C* and *D*, near *D*. The other is much darker, sharper, and broader, and lies midway between *D* and *E*. An absorption extends from these bands towards the red, terminating with a dark edge, which may be considered as a third band between the other two.

A dilute alkaline solution shows four bands, namely, a band between *C* and *D*; a second, broader band surrounding *D* and with the greater part between *D* and *E*; a third between *D* and *E*, nearly at *E*; and lastly, a fourth broad and dark band between *b* and *F*. On the addition of an alkaline zinc-chloride solution the spectrum changes more or less rapidly,[3] and finally a spectrum is obtained with only two bands, one of which surrounds *D* and the other lies between *D* and *E*. If an acid hæmatoporphyrin solution is shaken with chloroform, a part of the pigment is taken up by the chloroform, and this solution often shows a five-banded spectrum with two bands between *C* and *D*. The position of the hæmatoporphyrin bands in the spectrum differs with the various methods of preparation and other conditions, so that they do not correspond to the same wave-length. These facts coincide well with the recent investigations of A. SCHULZ;[4] according to which the appearance of the spectrum is not only dependent upon the reaction but also upon the character of the solvent and the method of preparation.

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[1] Zeitschr. f. physiol. Chem., 43.

[2] *Ibid.*, 43 and 45.

[3] See Hammarsten, Skand. Arch. f. Physiol., 3, and Garrod, Journ. of Physiol., 13.

[4] Arch. f. (Anat. u.) Physiol., 1904, Suppl.

In regard to the preparation of hæmatoporphyrin, see HOPPE-SEYLER-THIERFELDER's Handbuch, 7. Aufl., and the works cited on page 213.

Hæmatinogen is a ferruginous pigment so named by FREUND,[1] which he obtained by carefully extracting blood with alcohol containing hydrochloric acid. It is closely related to hæmatin, but is not sufficiently characteristic and is not considered as a cleavage product.

. A question of great interest is whether it is possible to produce the blood-pigment from its cleavage products. In this regard certain recent investigations are interesting. ZALESKI obtained from mesoporphyrin hydrochloride dissolved in 80 per cent acetic acid saturated with NaCl and heated to 50–70°, a hæmin-like pigment by the addition of a solution of iron in acetic acid, and this pigment had a spectrum in acid solution very similar to that of hæmatin, although not identical with it. ZALESKI considers this pigment as a hydrogenized hæmin. A regeneration of hæmin from hæmatoporphyrin has been performed by LAIDLAW. If hæmatoporphyrin is dissolved in dilute ammonia and warmed with STOKES' solution and hydrazine hydrate, iron is taken up again and hæmochromogen is produced, which is changed into hæmatin by shaking with air. According to HAM and BALEAN,[2] it is possible to produce hæmoglobin from hæmochromogen and globin, and it is indeed possible that other proteins can replace globin in this formation.

Hæmatoidin, thus called by VIRCHOW, is a pigment which crystallizes in orange-colored rhombic plates, and which occurs in old blood extravasations, and whose origin from the blood-coloring matters seems to be established (LANGHANS, CORDUA, QUINCKE, and others [3]). A solution of hæmatoidin shows no absorption-bands, but only a strong absorption from the violet to the green (EWALD [4]). According to most observers, hæmatoidin is identical with the bile-pigment bilirubin. It is not identical with the crystallizable lutein from the *corpora lutea* of the ovaries of the cow (PICCOLO and LIEBEN,[5] KÜHNE and EWALD).

In the detection of the above-described blood-coloring matters the spectroscope is the only entirely trustworthy means of investigation. If it is only necessary to test for blood in general and not to determine definitely whether the coloring-matter is hæmoglobin, methæmoglobin, or hæmatin, then the preparation of hæmin crystals is an absolutely positive test. The reader is referred to more extended text-books for more exact methods for the detection of blood in chemico-legal cases, and it is perhaps sufficient to give here the chief points of the investigation.

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[1] Wien. klin. Wochenschr., 1903.

[2] Zaleski, Zeitschr. f. physiol. Chem., **43**; Laidlaw, Journ. of Physiol., **31**; Ham and Balean, *ibid.*, **32**.

[3] A comprehensive review of the literature pertaining to hæmatoidin may be found in Stadelmann, Der Icterus, etc., Stuttgart, 1891, pp. 3 and 45.

[4] Zeitschr. f. Biologie, **22**, 475.

[5] Cit. from Gorup-Besanez, Lehrbuch d. physiol. Chem., 4. Aufl., 1878.

If spots on clothes, linen, wood, etc., are to be tested for the presence of blood, it is best, when possible, to scratch or shave off as much as possible, rub with common salt, and from this prepare the hæmin crystals. On obtaining positive results the presence of blood is not to be doubted. When sufficient material is not obtained by the above means, soak the spot with a few drops of water in a watch-crystal. If a colored solution is thus obtained, then remove the fibres, wood-shavings, and the like as far as possible, and allow the solution to dry in the watch-glass. The dried residue may be partly used for the spectroscopic test directly, and part may be employed in the preparation of the hæmin crystals. It may also be used to detect hæmochromogen in alkaline solution after previous treatment with alkali and the addition of reducing substances.

If a colorless solution is obtained after soaking with water, or if the spots are on rusty iron, then digest with a little dilute alkali (5 p. m.). In the presence of blood the solution gives, after neutralization with hydrochloric acid and drying, a residue which may give the hæmin crystals with glacial acetic acid. Another part of the alkaline solution shows, after the addition of STOKES' reduction fluid, the absorption-bands of hæmochromogen in alkaline solution.[1]

The methods proposed for the quantitative estimation of the blood-coloring matters are partly chemical and partly physical.

Among the chemical methods to be mentioned is the incineration of the blood and the determination of the amount of iron contained in the ash, from which the amount of hæmoglobin may be calculated. JOLLES[2] has recently suggested a clinical method based on this procedure.

The physical methods consist either of colorimetric or of spectroscopic investigations.

The principle of HOPPE-SEYLER'S *colorimetric method* is that a measured quantity of blood is diluted with an exactly measured quantity of water until the diluted blood solution has the same color as a pure oxyhæmoglobin solution of a known strength. The amount of coloring-matter present in the undiluted blood may be easily calculated from the degree of dilution. In the colorimetric testing we use a glass vessel with parallel sides containing a layer of liquid 1 cm. thick (HOPPE-SEYLER'S hæmatinometer). The use of HOPPE-SEYLER'S colorimetric double pipette is more advantageous. Other good forms of apparatus have been constructed by GIACOSA and ZANGERMEISTER.[3] Instead of an oxyhæmoglobin solution we now generally use a carbon-monoxide hæmoglobin solution as a standard liquid because it may be kept for a long time. The blood solution in this case is saturated with carbon monoxide.[1]

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[1] On the use of color reactions for the detection of blood, see O. and R. Adler, Zeitschr. f. physiol. Chem., 41, and Schumm and Westphal, *ibid.*, 46.

[2] Pflüger's Arch., 65; Monatshefte f. Chem., 17. See also Oerum, Zeitschr. f. anal. Chem., 43, and the works cited in Maly's Jahresber., 33.

[3] F. Hoppe-Seyler, Zeitschr. f. physiol. Chem., 16; G. Hoppe-Seyler, *ibid.*, 21; Winternitz, *ibid.*, Giacosa, Maly's Jahresber., 26; Zangermeister, Zeitschr. f. Biologie, 33.

The quantitative estimation of the blood-coloring matters by means of the spectroscope may be done in different ways, but at the present time the *spectrophotometric* method is chiefly used, and this seems to be the most reliable. This method is based on the fact that the extinction coefficient of a colored liquid for a certain region of the spectrum is directly proportional to the concentration, so that $C:E=C_1:E_1$, when C and C_1 represent the different concentrations and E and E_1 the corresponding coefficients of extinction. From the equation $\frac{C}{E}=\frac{C_1}{E_1}$, it follows that for one and the same pigment this relation, which is called the *absorption ratio*, must be constant. If the absorption ratio is represented by A, the determined extinction coefficient by E, and the concentration (the amount of coloring-matter in grams in 1 c.c.) by C, then $C=A\,.\,E$.

Different forms of apparatus have been constructed (Vierordt and Hüfner[2]) for the determination of the extinction coefficient, which is equal to the negative logarithm of those rays of light which remain after the passage of the light through a layer 1 cm. thick of an absorbing liquid. In regard to this apparatus the reader is referred to other text-books.

For purposes of control the extinction coefficients are determined in two different regions of the spectrum. Hüfner has selected (*a*) the region between the two absorption-bands of oxyhæmoglobin, especially between the wave-lengths 554 $\mu\mu$ and 565 $\mu\mu$, and (*b*) the region between the two bands, especially the interval between the wave-lengths 531.5 $\mu\mu$ and 542.5 $\mu\mu$. The constants or the absorption ratio for these two regions of the spectrum are designated by Hüfner by A and A'. Before the determination the blood must be diluted with water, and if the proportion of dilution of the blood be represented by V, then the concentration or the amount of coloring-matter in 100 parts of the undiluted blood is

$$C=100\,.\,V\,.\,A\,.\,E \text{ and}$$
$$C=100\,.\,V\,.\,A'\,.\,E'\,.$$

The absorption ratio or the constants in the two above-mentioned regions of the spectrum have been determined for oxyhæmoglobin, hæmoglobin, carbon monoxide hæmoglobin, and methæmoglobin, as follows:

Oxyhæmoglobin................$A_o=0.002070$ and $A'_o=0.001312$
Hæmoglobin.. ....$A_r=0.001354$ and $A'_r=0.001778$
Carbon-monoxide hæmoglobin...$A_c=0.001383$ and $A'_c=0.001263$
Methæmoglobin................$A_m=0.002077$ and $A'_m=0.001754$

The quantity of each coloring-matter may be determined in a mixture of two blood-coloring matters by this method; this is of special importance in the determination of the quantity of oxyhæmoglobin and hæmoglobin present in blood at the same time.

In order to facilitate these determinations, Hüfner[3] has worked out tables which give the relation between the two pigments existing in a solution containing oxyhæmoglobin and another pigment (hæmoglobin, methæmoglobin, or carbonmonoxide hæmoglobin), and thus allowing of the calculation of the absolute quantity of each pigment.

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[1] See Haldane, Journ. of Physiol., **26**.

[2] See Vierordt, Die Anwendung des Spektralapparates zu Photometrie, etc. (Tübingen, 1873), and Hüfner, Arch. f. (Anat. u.) Physiol., 1894, and Zeitschr. f. physiol. Chem., **3**; v Noorden, *ibid.*, **4**; Otto, Pflüger's Arch., **31** and **36**.

[3] Arch. f. (Anat. u.) Physiol., 1900.

Among the many apparatuses constructed for clinical purposes for the quantitative estimation of hæmoglobin, Fleischl's *hæmometer*, which has undergone numerous modifications, Henocque's *hæmatoscope*, and Sahli's *hæmometer* are to be specially mentioned. In regard to these apparatuses, see v. Jaksch, Klinische Diagnostik innerer Krankheiten, 4. Auflage, 1897, and Jaquet, Korresp.-Blatt f. Schweiz. Aerzte, 1897; Gärtner, Münchener med. Wochenschr., 1901, and H. Sahli, Diagnostic Methods, Philadelphia, 1905.

Many other pigments are found besides the often-occurring hæmoglobin in the blood of invertebrates. In a few Arachnidæ, Crustacea, Gasteropodæ, and Cephalopodæ a body analogous to hæmoglobin, containing copper, *hæmocyanin*, has been found by Fredericq. By the taking up of loosely bound oxygen this body is converted into blue *oxyhæmocyanin*, and by the escape of the oxygen becomes colorless again. According to Henze 1 gram hæmocyanin combines with about 0.4 c.c. oxygen. It is crystalline and has the following composition: C 53.66; H 7.33; N 16.09; S 0.86; Cu 0.38; O 21.67 per cent. On hydrolytic cleavage with hydrochloric acid Henze found the following division of the nitrogen in hæmocyanin: Of the total nitrogen 5.78 per cent was split off as ammonia, 2.67 per cent as humus nitrogen, 27.65 per cent as diamino nitrogen, and 63.39 per cent as monamino nitrogen. He found no arginine in the cleavage products, but could detect histidine, lysine, tyrosine, and glutamic acid. A coloring-matter called *chlorocruorin* by Lankester is found in certain Chætopodæ. *Hæmerythrin*, so called by Krukenberg but first observed by Schwalbe, is a red coloring-matter from certain Gephyrea. Besides hæmocyanin we find in the blood of certain Crustacea the red coloring-matter *tetronerythrin* (Halliburton), which is also widely spread in the animal kingdom. *Echinochrom*, so named by MacMunn,[1] is a brown coloring-matter occurring in the perivisceral fluid of a variety of echinoderms.

The *quantitative constitution of the red blood-corpuscles*. The amount of water varies in different varieties of blood-corpuscles between 570–644 p. m., with a corresponding amount, 430–356 p. m., of solids. The chief mass, about $\frac{8}{10}$–$\frac{9}{10}$, of the dried substance consists of hæmoglobin (in human and mammalian blood).

According to the analyses of Hoppe-Seyler[2] and his pupils, the red corpuscles contain in 1000 parts of the dried substance:

	Hæmoglobin	Protein	Lecithin	Cholesterin
Human blood..........	868–944	122–51	7.2–3.5	2.5
Dog's "	865	126	5.9	3.6
Goose's "	627	364	4.6	4.8
Snake's "	467	525	...	...

Abderhalden found the following composition for the blood-corpuscles from the domestic animals investigated by him: Water, 591.9–644.3

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[1] Fredericq, Extrait des Bulletins de l'Acad. Roy. de Belgique (2), 46, 1878; Lankester, Journ. of Anat. and Physiol., 2 and 4; Henze, Zeitschr. f. physiol. Chem., 33 and 43; Krukenberg, see Vergl. physiol. Studien, Reihe 1, Abt. 3, Heidelberg, 1880; Halliburton, Journal of Physiol., 6; MacMunn, Quart. Journ. Microsc. Science, 1885.

[2] Med.-chem. Untersuch., 390 and 393.

p. m.; solids 408.1–335.7 p. m.; hæmoglobin, 303.3–331.9 p. m.; protein, 5.32 (dog)-78.5 p. m. (sheep); cholesterin, 0.388 (horse)-3.593 p. m. (sheep); and lecithin, 2.296 (dog)–4.855 p. m.

Of special interest is the varying proportion of the hæmoglobin to the protein in the nucleated and in the non-nucleated blood-corpuscles. These last are much richer in hæmoglobin and poorer in protein than the others.

The amount of mineral bodies in various species of animals is different. According to BUNGE and ABDERHALDEN the red corpuscles from the pig, horse, and rabbit contain no soda, while those from man, the ox, sheep, goat, dog, and cat are relatively rich in soda. In the five last-mentioned species the amount of soda was 2.135–2.856 p. m. The quantity of potash was 0.257 (dog)–0.744 p. m. (sheep). In the horse, pig, and rabbit the quantity of potash was 3.326 (horse)-5.229 p. m. (rabbit). Human blood-corpuscles contain, according to WANACH, about five times as much potash as soda, on an average 3.99 p. m. potash and 0.75 p. m. soda. The nucleated erythrocytes of the frog, toad, and turtle contain, according to BOTTAZZI and CAPPELLI,[1] also considerably more potassium than sodium. Lime is claimed to be absent in the blood-corpuscles, and magnesia occurs only in small amounts: 0.016 (sheep)–0.150 p. m. (pig). The blood-corpuscles of all animals investigated contain chlorine, 0.460–1.949 p. m. (both in horse), generally 1 to 2 p. m., and also phosphoric acid. The amount of inorganic phosphoric acid shows great variation: 0.275 (sheep)-1.916 p. m. (horse). All of the above figures are calculated on the fresh, moist blood-corpuscles.

By quantitative determinations of the swelling and shrinking of the cells under the influence of NaCl solutions of various concentration or of serum of various dilutions, HAMBURGER has attempted to determine for the erythrocytes, as well as the leucocytes, the percentage relationship between the two chief constituents of the cells (the frame and the intracellular fluid). He found that the volume of the frame-substance for both varieties of blood-corpuscles of the horse was equal to 53–56.1 per cent. The volume for the red blood-corpuscles was for the rabbit 48.7–51; hen, 52.4–57.7, and for the frog, 72–76.4 per cent. KOEPPE has raised objections to these determinations.[2]

The White Blood-corpuscles and the Blood-plates.

The White Blood-corpuscles, also called LEUCOCYTES or Lymphoid Cells, are of different kinds, and ordinarily we differentiate between the small forms poor in protoplasm, called lymphocytes, and the larger, granular, often more nucleated forms, called leucocytes. The polynuclear leucocytes occur in greater abundance in the blood than the lymphocytes.

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[1] Bunge, Zeitschr. f. Biologie, 12, and Abderhalden, Zeitschr. f. physiol. Chem., 23 and 25; Wanach, Maly's Jahresber., 18, 88; Bottazzi and Cappelli, Arch. Ital. de Biologie, 32.

[2] Hamburger, Arch. f. (Anat. u.) Physiol., 1898; Koeppe, *ibid.*, 1899 and 1900.

In human and mammalian blood, most of the white blood-corpuscles are larger than the red blood-corpuscles. They have also a lower specific gravity than the red corpuscles, move in the circulating blood nearer to the walls of the blood-vessels, and have also a slower motion.

The number of white blood-corpuscles varies not only in the different blood-vessels, but also under different physiological conditions. On an average there is only 1 white corpuscle for 350-500 red corpuscles. According to the investigations of ALEX. SCHMIDT[1] and his pupils, the leucocytes are destroyed in great part on the discharge of the blood before and during coagulation, so that discharged blood is much poorer in leucocytes than the circulating blood. The correctness of this statement has been denied by other investigators.

From a histological standpoint we generally, as above indicated, discriminate between the different kinds of colorless blood-corpuscles. Chemically considered, however, there is no known essential difference between them, and what little we do know chemically is chiefly in connection with the leucocytes. With regard to their importance in the coagulation of fibrin, ALEX. SCHMIDT and his pupils distinguish between the leucocytes which are destroyed in the coagulation and those which are not. The last mentioned give with alkalies or common-salt solutions a slimy mass; the first do not show such behavior.

The protoplasm of the leucocytes has during life amœboid movements which serve partly to make possible the wandering of the cells, and partly to aid in the absorption of smaller grains or foreign bodies. On these grounds the occurrence of *myosin* in them has been admitted even without any special proof thereof. ALEX. SCHMIDT claims to have found *serglobulin* in equine-blood leucocytes which have been washed with ice-cold water. There are also certain leucocytes, as above stated, which yield a slimy mass when treated with alkalies or NaCl solutions, which seems to be identical with the so-called *hyaline substance* of ROVIDA found in the pus-cells. On digesting the leucocytes with water, a solution of a protein body is obtained which can be precipitated by acetic acid and which forms the chief mass of the leucocytes. This substance, which is undoubtedly concerned in the coagulation of the blood, has been described under different names (see Chapter V, page 141), and consists, chiefly, at least, of nucleoproteid. The ordinary view that this is nucleohistone does not seem to be correct, according to the recent investigations of BANG,[2] and further proof is necessary.

Glycogen, as previously stated, is found in the leucocytes. The glycogen found by HUPPERT, CZERNY, DASTRE,[3] and others in blood and lymph probably originated from the leucocytes. A glucothionic acid has been prepared from white cells by MANDEL and LEVENE.[1] The constituents of the leucocytes are the same as the constituents of the cell as described in Chapter V.

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[1] Pflüger's Arch., **11**. Also Fr. Krüger, Arch. f. exp. Path. u. Pharm., **51**.

[2] I. Bang, Studier over Nukleoproteider, Kristiania, 1902.

[3] Huppert, Centralbl. f. Physiol., **6**, 394; Czerny, Arch. f. exp. Path. u. Pharm., **31**;

The **blood-plates** (BIZZOZERO), hæmatoblasts (HAYEM), whose nature, preformed occurrence, and physiological importance have been much questioned, are pale, colorless, gummy disks, round or somewhat oval in shape and generally with a diameter two or three times smaller than the red blood-corpuscles. By the action of different reagents the blood-plates separate into two substances, one of which is homogeneous and non-refractive, while the other is highly refractive and granular. Blood-plates readily stick together and attach themselves to foreign bodies.

According to the researches of KOSSEL and of LILIENFELD[2] the blood-plates consist of a chemical combination between protein and nuclein, and hence they are also called *nuclein-plates* by LILIENFELD, and are considered as derivatives of the cell nucleus. It seems certain that the blood-plates have some connection with the coagulation of blood. The views on this question and especially in regard to the manner in which these plates act in coagulation are unfortunately very divergent.

III. THE BLOOD AS A MIXTURE OF PLASMA AND BLOOD-CORPUSCLES.

The blood in itself is a thick, sticky, light or dark red liquid, opaque even in thin layers, having a salty taste and a faint odor differing in different kinds of animals. On the addition of sulphuric acid to the blood the odor is more pronounced. In adult human beings the specific gravity ranges between 1.045 and 1.075. It has an average of 1.058 for grown men and a little less for women. LLOYD JONES found that the specific gravity is highest at birth and lowest in children when about two years old and in pregnant women. The determinations of LLOYD JONES, HAMMERSCHLAG,[3] and others show that the variation of the specific gravity, dependent upon age and sex, corresponds to the variation in the quantity of hæmoglobin.

The determination of the specific gravity is most accurately done by means of the pyknometer. For clinical purposes, where only small amounts

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Dastre, Compt. rend., **120**, and Arch. de Physiol. (5), **7**. See also Hirschberg, Zeitschr. f. klin. Med., **54**.

[1] Biochem. Zeitschr., **4**.

[2] In regard to the literature of the blood-plates, see Lilienfeld, Arch. f. (Anat. u.) Physiol., 1892, and "Leukocyten und Blutgerinnung," Verhandl. d. physiol. Gesellsch. zu Berlin, 1892; and also Mosen, Arch. f (Anat. u.) Physiol., 1893, and Maly's Jahresber., **20** and **21**.

[3] Lloyd Jones, Journ. of Physiol., 8; Hammerschlag, Wien. klin. Wochenschrift, **1890**, and Zeitschr f. klin. Med., **20**.

are available, it is best to proceed with the method as suggested by HAMMERSCHLAG. Prepare a mixture of chloroform and benzene of about 1.050 sp. gr. and add a drop of the blood to this mixture. If the drop rises to the surface then add benzene, and if it sinks add chloroform. Continue this until the drop of blood suspends itself midway and then determine the specific gravity of the mixture by means of an areometer. This method is not strictly accurate and must be performed quickly. In regard to the necessary details refer to ZUNTZ and A. LEVY.[1]

The reaction of the blood is alkaline towards litmus. The quantity of alkali, calculated as Na_2CO_3, in fresh, non-defibrinated blood from the dog, horse, and man, is, according to LOEWY, 4.93, 4.43, and 5.95 p. m. respectively. According to STRAUSS the average for normal human blood may be calculated as about 4.3 p. m. Na_2CO_3. Quantities below 3.3 p. m. and above 5.3 p. m. are, according to him, to be considered as pathological. v. JAKSCH found the quantity of alkali in man to vary between 3.38 and 3.90 p. m., and BRANDENBURG found 3 p. m. NaOH(=3.98 p. m. Na_2CO_3). The alkaline reaction diminishes outside of the body, and indeed the more quickly the greater the original alkalinity of the blood. This depends on the formation of acid in the blood, in which the red blood-corpuscles seem to take part in some way or another. After excessive muscular activity the alkalinity is diminished (PEIPER, COHNSTEIN), and it is also decreased after the continuous ingestion of acids (LASSAR, FREUDBERG[2]). Numerous investigations have been made in regard to the alkalinity of the blood in disease, but as there is at present no trustworthy method for estimating the alkalinity of the blood, and as the results are dependent upon the indicator used, these investigations, as also the statements in regard to the physiological alkalinity, require further substantiation.[3] Attention must also be called to what was stated (page 191) in regard to the determination of the alkalinity of blood-serum—that determinations are made only of the titratable alkali and not of the true alkalinity caused by hydroxyl ions.

The alkali of the blood exists in part as alkaline salts, carbonate and phosphate, and partly in combination with protein or hæmoglobin. The first are often spoken of as readily diffusible alkalies, while the others are not, or are only diffusible with difficulty (see page 187). The quantity of the first in human blood is about one fifth of the total alkali (BRANDENBURG). The readily as well as the difficultly diffusible alkali is divided between the blood-corpuscles and plasma, and the blood-corpuscles seem to be richer in difficultly diffusible alkali than the plasma or serum. This division may be changed by the influence of even very small amounts of acid, even of carbonic acid, and also, as shown by ZUNTZ, LOEWY and ZUNTZ, HAMBURGER, LIMBECK, and GÜRBER,[1] by the influence of the respiratory exchange of gas. The blood-corpuscles give up a part of the alkali united with protein to the serum by the action of carbon dioxide, hence the serum becomes more alkaline. The equilibrium of the osmotic tension in the blood-corpuscles and in the serum is hereby destroyed; the blood-corpuscles swell up because they take up water from the serum, and this then becomes more concentrated and richer in alkali, protein, and sugar. Under the influence of oxygen, the corpuscles take their original form again and the above changes are reversed. The blood-corpuscles for this reason are less biconcave in their small diameter in venous than in arterial blood (HAMBURGER).

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[1] Zuntz, Pflüger's Arch., **66**; Levy, Proceed. Roy. Soc., **81**.

[2] Loewy, Pflüger's Arch., **58**, which also contains the references to the literature; H. Strauss, Zeitschr. f. klin. Med., **30**; v. Jaksch, *ibid.*, **13**; Peiper, Virchow's Arch., **116**; Cohnstein, *ibid.*, **130**, which also cites the works of Minkowski, Zuntz, and Geppert; Freudberg, *ibid.*, **125**. See also Weiss, Zeitschr. f. physiol. Chem., **38**; Brandenburg, Zeitschr f. klin. Med., **45**.

[3] In regard to the methods for the estimation of the alkalinity see, besides the above-mentioned authors, v. Jaksch, Klin. Diagnostik; v. Limbeck, Wien. med. Blätter, **18**; Wright, The Lancet, 1897; Biernacki, Beiträge zur Pneumatologie, etc., Zeitschr. f. klin. Med., **31** and **32**; Hamburger, Eine Methode zur Trennung, etc., Arch f. (Anat. u.) Physiol, 1898. See also Maly's Jahresber., **29**, **30**, and **31**; Salaskin and Pupkin, Zeitschr. f. physiol. Chem., **42**, and O. Folin, *ibid.*, **43**.

These conditions have been further studied by v. KORANYI and BENCE,[2] and they have investigated the relationship between the changes of the volume of the blood-corpuscles and the electrical conductivity, the refractivity of the serum and the viscosity of the blood. The refraction coefficient of the serum is highest with an increase in the amount of carbon dioxide, while it is lowest when the blood is rich in oxygen and poor in carbon dioxide. They consider this as an action of acid, as a similar rise is observed after the addition of acid, while after the addition of alkali a fall in the refraction coefficient of the serum takes place, and these same changes can be brought about by CO_2 or by a current of oxygen. With an increase in the amount of carbon dioxide, the conductivity of the blood diminishes; the viscosity is, on the other hand, highest when the blood is richest in carbon dioxide. If the CO_2 is driven off by O the viscosity diminishes to a minimum, and on leading in more oxygen it rises again. The changes in viscosity of the blood run parallel with the volume changes of the blood-corpuscles, and changes in the viscosity, which can be brought about by the removal of carbon dioxide, cause a change in the electric charge of the blood-corpuscles (v. KORANYI and BENCE).

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[1] Zuntz in Hermann's Handbuch der Physiol., **4**, Abt. 2; Loewy and Zuntz, Pflüger's Arch., **58**; Hamburger, Arch. f. (Anat. u.) Physiol., 1894 and 1898, and Zeitschr. f. Biologie, **28** and **35**; v. Limbeck, Arch. f. exp. Path. u. Pharm., **35**; Gürber, Sitzungsber. d. phys. med. Gesellsch. zu Würzburg, 1895.

[2] Pflüger's Arch., **110**.

The color of the blood is red—light scarlet-red in the arteries and dark bluish red in the veins. Blood free from oxygen is dichroitic, dark red by reflected light and green by transmitted light. The blood-coloring matters occur in the blood-corpuscles. For this reason blood is opaque in thin layers. If the hæmoglobin is removed from the stroma and dissolved by the blood liquid by any of the above-mentioned means (see page 193), the blood becomes transparent and has then a "*lake color.*"[1] Less light is now reflected from its interior, and this laky blood is therefore darker in thicker layers. On the addition of salt solutions to the blood-corpuscles they shrink, more light is reflected, and the color appears lighter. A great abundance of red corpuscles makes the blood darker, while by diluting with serum or by a greater abundance of white corpuscles the blood becomes lighter in appearance. The different colors of arterial and of venous blood depend on the varying quantities of gas contained in these two varieties of blood, or, better, on the different amounts of oxyhæmoglobin and hæmoglobin they contain.

The most striking property of blood consists in its coagulating within a shorter or longer time, but as a rule very shortly after leaving the veins. Different kinds of blood coagulate with varying rapidity; in human blood the first marked sign of coagulation is seen in two to three minutes, and within seven to eight minutes the blood is thoroughly converted into a gelatinous mass. If the blood is allowed to coagulate slowly, the red corpuscles have time to settle more or less before the coagulation, and the blood-clot then shows an upper yellowish-gray or reddish-gray layer consisting of fibrin enclosing chiefly colorless corpuscles. This layer has been called *crusta inflammatoria* or *phlogistica*, because it has been especially observed in inflammatory processes and is considered one of the characteristics of them. This crusta, or "*buffy coat,*" is not characteristic of any special disease and it occurs chiefly when the blood coagulates slowly or when the blood-corpuscles settle more quickly than usual. A buffy coat is often observed in the slowly coagulating equine blood. The blood from the capillaries is not supposed to have the power of coagulating.

Coagulation is retarded by cooling, by diminishing the oxygen, and by increasing the amount of carbon dioxide, which is the reason that venous blood and to a much higher degree blood after asphyxiation coagulates more slowly than arterial blood. The coagulation may be retarded or prevented by the addition of acids, alkalies, or ammonia, even in small quantities; by concentrated solutions of neutral alkali salts and alkaline earths, alkali oxalates and fluorides; also by egg-albumin solutions of sugar or gum, glycerine, or much water; also by receiving the blood in oil. Coagulation may be prevented by the injection of a proteose solution or of an infusion of the leech into the circulating blood, but this infusion also acts in the same way on blood just drawn. Coagulation is also hindered by snake poison and toxines (see page 171). The coagulation may be facilitated by raising the temperature; by contact with foreign bodies, to which the blood adheres; by stirring or beating it; by admission of air; by diluting with very small amounts of water; by the addition of platinum-black or finely powdered carbon; by the addition of laky blood, which does not act by the presence of dissolved blood-coloring matters, but by the stromata of the blood-corpuscles; and also by the addition of the leucocytes from the lymphatic glands, or of a watery saline extract of the lymphatic glands, testicles, or thymus and various other organs (DELEZENNE, WRIGHT, ARTHUS,[1] and others).

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[1] R. Du Bois-Reymond presents objections to the general use of the above terms in Centralbl. f. Physiol., 19, p. 65.

An important question to answer is why the blood remains fluid in the circulation, while it quickly coagulates when it leaves the circulation. The reason why blood coagulates on leaving the body is therefore to be sought for in the influence which the walls of the living and uninjured blood-vessels exert upon it. These views are derived from the observations of many investigators. From the observations of HEWSON, LISTER, and FREDERICQ it is known that when a vein full of blood is ligatured at the two ends and removed from the body, the blood may remain fluid for a long time. BRÜCKE[2] allowed the heart removed from a tortoise to beat at 0° C., and found that the blood remained uncoagulated for some days. The blood from another heart quickly coagulated when collected over mercury. In a dead heart, as also in a dead blood-vessel, the blood soon coagulates, and also when the walls of the vessel are changed by pathological processes.

What then is the influence which the walls of the vessels exert on the liquidity of the circulating blood? FREUND has found that the blood remains fluid when collected by means of a greased canula under oil or in a vessel smeared with vaseline. If the blood collected in a greased vessel be beaten with a glass rod previously oiled, it does not coagulate, but it quickly coagulates on beating it with an unoiled glass rod or when it is poured into a vessel not greased. The non-coagulability of blood collected under oil was confirmed later by HAYCRAFT and CARLIER. FREUND found on further investigation that the evaporation of the upper layers of blood or their contamination with small quantities of dust causes a coagulation even in a vessel treated with vaseline. According to FREUND[1] it is this adhesion between the blood or, as the blood shows an adhesion to the normal vessel walls (BENNO LEWY), between its form-elements and a foreign substance—and the diseased walls of the vessel also act as such—that gives the impulse towards coagulation, while the lack of adhesion prevents the blood from coagulating. BORDET and GENGOU[2] have also shown that the plasma obtained by centrifuging blood collected in a paraffined vessel, and perfectly free from form-elements, can be kept without coagulating in a paraffined vessel, and that it does coagulate on being transferred to an unparaffined vessel. The adhesion of the plasma to a foreign body may also, in the absence of form-elements, give the impulse to coagulation. That this adhesion of the form-elements is of great importance cannot be denied and is also generally accepted. By this adhesion the form-elements undergo certain changes which seem to stand in a certain relationship to the coagulation of the blood.

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[1] Delezenne, Arch. de Physiol. (5), 8; Wright, Journ. of Physiol., 28; Arthus, Journ. de Physiol. et Pathol., 4.

[2] Hewson's works, edited by Gulliver, London, 1876, cited from Gamgee, Text-book of Physiol. Chem., 1, 1880; Lister, cited from Gamgee, *ibid.*; Fredericq, Recherches sur la constitution du plasma sanguin, Gand, 1878; Brücke, Virchow's Arch., 12.

The views in regard to these changes are, unfortunately, very contradictory. According to ALEX. SCHMIDT[3] and the Dorpat school an abundant destruction of the leucocytes, especially polynuclear leucocytes, takes place in coagulation and important constituents for the coagulation of the fibrin pass into the plasma. A direct relationship between the destruction of leucocytes and coagulation is denied by many investigators, while according to other experimenters the essential is not a destruction of the leucocytes, but an elimination of constituents from the cells into the plasma. This process is called *plasmoschisis* by LÖWIT.[4] The passage of cell constituents into the plasma before coagulation must not necessarily be considered as a phenomenon of death, as it may just as well be a secretory process (ARTHUS, MORAWITZ, DASTRE[5]). Great importance has also been ascribed to the blood-platelets in coagulation, as certain investigators (BIZZOZERO, LILIENFELD, SCHWALBE, MORAWITZ, BÜRKER) have found that they cause or accelerate coagulation, while others (PETRONE) on the contrary find a retarding action.[6]

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[1] Freund, Wien. med. Jahrb., 1886; Haycraft and Carlier, Journ. of Anat. and Physiol., 22; Benno Lewy, Arch. f. (Anat. u.) Physiol., 1899, Suppl.

[2] Annal. de l'Institute Pasteur, 17.

[3] Pflüger's Arch., 11. The works of Alex. Schmidt are found in Arch. f. Anat. und Physiol., 1861, 1862; Pflüger's Arch., 6, 9, 11, 13. See especially Alex. Schmidt, Zur Blutlehre (Leipzig, 1892), which also gives the work of his pupils, and Weitere Beiträge zur Blutlehre, 1895.

[4] Wien. Sitzungsber., 89 and 90, and Prager med. Wochenschr., 1889, referred to in Centralbl. f. d. med. Wissensch., 28, 265.

[5] Morawitz, Hofmeister's Beiträge, 5; Arthus, Compt. rend. soc. biolog., 55; Dastre, ibid., 55.

[6] See foot-note 2, p. 222. Also Schwalbe, Unters. z. Blutgerinnung, etc., Braunschweig, 1900; Morawitz, Deutsch. Arch. f. klin. Med., 79, and Hofmeister's Beiträge, 4 and 5; Bürker, Pflüger's Arch., 102, Petrone, Maly's Jahresber., 31, p. 170.

WOOLDRIDGE[1] takes a very peculiar position in regard to this question: he considers the form-elements as only of secondary importance in coagulation. As he has found, a peptone-plasma which has been freed from all form-constituents by means of centrifugal force yields abundant fibrin when it is not separated from a substance which precipitates on cooling. This substance, which WOOLDRIDGE has called A-fibrinogen, seems to all appearances to be a nucleoproteid, which, according to the unanimous view of several investigators, originates from the form-elements of the blood, either the blood-plates or the leucocytes, and the generally accepted view as to the great importance of the form-elements in the coagulation of the blood is not really contrary to WOOLDRIDGE's experiments.

The views are greatly divided in regard to those bodies which are eliminated from the form-elements of the blood before and during coagulation.

According to ALEX. SCHMIDT the leucocytes, like all cells, contain two chief groups of constituents, one of which accelerates coagulation, while the other retards or hinders it. The first may be extracted from the cells by alcohol, while the other cannot be extracted. Blood-plasma contains only traces of thrombin, according to SCHMIDT, but does contain its antecedent, prothrombin. The bodies which accelerate coagulation are neither thrombin nor prothrombin, but they act in this wise in that they split off thrombin from the prothrombin. On this account they are called *zymoplastic substances* by ALEX. SCHMIDT. The nature of these bodies is unknown, and SCHMIDT has given no notice of their behavior with the lime salts, which have been found to have zymoplastic activity by other investigators.

The constituents of the cells which hinder coagulation and which are insoluble in alcohol-ether are compound proteids and have been called *cytoglobin* and *preglobulin* by SCHMIDT. The retarding action of these bodies may be suppressed by the addition of zymoplastic substances, and the yield of fibrin on coagulation in this case is much greater than in the absence of the compound proteid retarding coagulation. This last supplies the material from which the fibrin is produced. The process is, according to SCHMIDT, as follows: The preglobulin first splits, yielding serglobulin, then from this the fibrinogen is derived, and from this latter the fibrin is produced. The object of the thrombin is twofold. The thrombin first splits the fibrinogen from the paraglobulin and then converts the fibrinogen into fibrin. The assumption that fibrinogen can be split from paraglobulin has not sufficient foundation and is even improbable.

According to SCHMIDT the retarding action of the cells is prominent during life, while the accelerating action is especially pronounced outside of the body or by coming in contact with foreign bodies. The parenchymous masses of the organs and tissues, through which the blood flows in the capillaries, are those cell-masses which serve to keep the blood fluid during life.

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[1] Die Gerinnung des Blutes (published by M. v. Frey, Leipzig, 1891).

LILIENFELD has given further proof as to the occurrence in the form-elements of the blood of bodies which accelerate or retard the coagulation. According to this author the nature of these bodies is very markedly different from SCHMIDT'S idea. While, according to SCHMIDT, the coagulation accelerators are bodies soluble in alcohol, and the compound proteids exhausted with alcohol act only retardingly on coagulation, LILIENFELD states that the substance which acts acceleratingly and retardingly on coagulation consists of a nucleoproteid, namely, nucleohistone. Nucleohistone readily splits into leuconuclein and histone, the first of which acts as a coagulation-excitant, while the other, introduced into the blood-vascular system, either intravascular or extravascular, robs the blood of its property of coagulating. Introduced into the circulatory system the nucleohistone splits into its two components. It therefore causes extensive coagulation on one side and makes the remainder of the blood uncoagulable on the other. This theory as well as that of SCHMIDT is not based upon sufficiently positive facts.

BRÜCKE showed long ago that fibrin left an ash containing calcium phosphate. The fact that calcium salts may facilitate or even cause a coagulation in liquids poor in ferment has been known for several years through the researches of HAMMARSTEN, GREEN, RINGER and SAINSBURY. The necessity of the lime salts for the coagulation of blood and plasma was first shown positively by the important investigations of ARTHUS and PAGÈS. Recent investigations of SABBATANI[1] have also shown the importance of calcium salts or the free calcium ions for coagulation without explaining the mode of their action.

According to the generally accepted view of ARTHUS and PAGÈS the soluble lime salts precipitable by oxalate are necessary requisites for the fermentive transformation of fibrinogen, because thrombin remains inactive in the absence of soluble lime salts. This view is untenable, as shown by the researches of ALEX. SCHMIDT, PEKELHARING, and HAMMARSTEN.[2] Thrombin acts as well in the absence as in the presence of precipitable lime salts.

LILIENFELD'S theory that the leuconuclein splits off a protein substance, *thrombosin*, from the fibrinogen, and that this thrombosin forms an insoluble compound with the lime present, producing thrombosin lime (fibrin), which separates, is incorrect according to HAMMARSTEN, SCHÄFER, and CRAMER.[3] LILIENFELD'S thrombosin is nothing but fibrinogen which is precipitated by a lime salt from a salt-poor or salt-free solution.

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[1] Hammarsten, Nova Acta reg. Soc. Scient. Upsal. (3), 10, 1879; Green, Journ. of Physiol., 8; Ringer and Sainsbury, *ibid.*, 11 and 12; Arthus et Pagès and Arthus, see foot-note 4, p. 171; Hammarsten, Zeitschr. f. physiol. Chem., 22; Sabbatani, cited, Centralbl. f. Physiol., 16, 665.

[2] Hammarsten, Zeitschr. f. physiol. Chem., 22, where the other investigators are cited.

[3] Hammarsten, l. c.; Schäfer, Journ. of Physiol., 17; Cramer, Zeitschr. f. physiol. Chem., 23.

According to PEKELHARING[1] thrombin is the lime compound of prothrombin, and the process of coagulation consists, according to him, in the thrombin transferring the lime to the fibrinogen, which is hereby converted into an insoluble lime compound, fibrin. Of the objections to this theory can be mentioned, among others, the fact that fibrin has been obtained not absolutely free from lime, but still so poor in lime (HAMMARSTEN[2]) that if the lime belongs to the fibrin, its molecule must be more than ten times greater than the hæmoglobin molecule, which is not probable. These as well as many other observations indicate that the lime is carried down by the fibrinogen only as a contamination.

If, as it seems, the lime is not of importance in the transformation of fibrinogen into fibrin in the presence of thrombin, still this does not contradict the above-mentioned observations of ARTHUS and PAGÈS that the lime salts are necessary for the coagulation of blood and plasma. It is very probable that the lime salts, as admitted by PEKELHARING, are a necessary requisite for the transformation of prothrombin into thrombin.

If we attempt to summarize the more or less contradictory investigations and views as given in the preceding pages, we can consider the following facts as conclusive: In the first place, two bodies, the fibrinogen and the thrombin, are necessary for the coagulation. The fibrinogen exists preformed in the plasma. The thrombin, on the contrary, does not occur in living blood, at least not in appreciable amounts as such, but is formed from another substance, the prothrombin. The presence of calcium salts is necessary for the formation of this thrombin, while the calcium salts are not necessary for the enzymotic transformation of fibrinogen into fibrin. Besides the calcium salts also other substances, the zymoplastic active substances, are active in the formation of thrombin from its mother-substance, and these zymoplastic substances stand in some relation to the form-elements of the blood.

The formation of thrombin and the relationship of the form-elements therewith are still unexplained or disputed questions.

It is a question whether the mother-substance of thrombin exists in the plasma of the circulating blood or whether it is a body eliminated from the form-elements before coagulation. We have two contradictory views on this question, namely, those of ALEX. SCHMIDT and of PEKELHARING. According to SCHMIDT prothrombin occurs preformed in the circulating plasma, and it is transformed into thrombin by the zymoplastic substances which pass out from the form-elements. PEKELHARING, on the contrary, holds the view that the plasma does not contain appreciable amounts of prothrombin. This body, according to him, passes before coagulation from the form-elements into the plasma, and is there converted into thrombin by the calcium salts. The observation that uncoagulated leech-plasma does not coagulate on the addition of calcium salts, while it does coagulate on the addition of prothrombin solutions, seems to support this view; still it is not quite conclusivé. Leech-extract contains a body, hirudin, which, according to MORAWITZ, is an antibody towards thrombin and quantitatively neutralizes it. On the addition of prothrombin, new thrombin may be formed, which may act if the hirudin is not present in too great an excess.

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[1] See foot-note 6, p. 175, and especially Virchow's Festschrift, **1**, 1891.

[2] Zeitschr. f. physiol. Chem., 28.

The behavior of sodium-fluoride plasma shows more conclusively the absence of prothrombin in the circulating plasma. Such plasma, according to ARTHUS, contains no prothrombin, a statement which has been partly substantiated by MORAWITZ, who finds that fluoride-plasma contains more or less prothrombin, dependent upon the greater or less change the blood undergoes before it flows into the sodium-fluoride solution. One can obtain, according to MORAWITZ, at least sometimes, a fluoride-plasma which contains no prothrombin. The observations of FULD and of SCHITTENHELM and BODONG contradict the statement that fluoride-plasma contains prothrombin. As BORDET and GENGOU [1] have shown that prothrombin can be carried down by the precipitate produced in fluoride-plasma, it seems as if the observations of ARTHUS and MORAWITZ on this point are not conclusive, and it is probable that all plasma contains prothrombin. The absence of prothrombin, as observed by ARTHUS, in peritoneal transudates in the horse, can hardly be considered as sufficient evidence as to the occurrence of this body in blood-plasma.

The unsettled condition of the question of the zymoplastic substances has recently been somewhat enlightened, and the starting-point in these new investigations is the accelerating action upon coagulation, of different tissue extracts, an action which has been known for a long time and was especially studied by DELEZENNE on the plasma from bird's blood. The active constituent of these tissue extracts is called *thrombokinase* by MORAWITZ, and, according to him, this thrombokinase is necessary, besides lime-salts, for the transformation of prothrombin (*thrombogen* according to MORAWITZ). The production of thrombokinase is, according to MORAWITZ, a general property of the protoplasm and occurs also in the leucocytes. The thrombokinase of drawn blood originates in birds and in part in mammals from the leucocytes. In mammalian blood the blood-plates are the essential source. For the formation of thrombin three different substances are necessary according to MORAWITZ, namely, thrombogen, thrombokinase, and lime-salts. *Thrombogen* is, according to MORAWITZ, not quite identical with the prothrombin (other investigators), which he calls α-prothrombin, but is a mother-substance of the same. The process of thrombin formation can be given as follows: the kinase first transforms the thrombogen into α-prothrombin, which latter then is converted into thrombin (α) by the lime-salts.

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[1] Arthus, Journ. de Physiol. et Pathol., 3 and 4, and Compt. rend. soc. biol., 56. The works of Morawitz may be found in Hofmeister's Beiträge, 4 and 5, Deutsch. Arch. f. klin. Med., 79 and 80, and Centralbl. f. Physiol., 17, p. 529; with Spiro, Hofmeister's Beiträge, 5; Schittenhelm and Bodong, Arch. f. exp. Path. u. Pharm., 54; Bordet and Gengou, Annal. Institut Pasteur, 18.

Thrombokinase is precipitated by alcohol and is not resistant towards heat. It therefore cannot be identical with SCHMIDT's zymoplastic substances, and this point requires further elucidation. The thrombokinase also does not occur to any appreciable extent in the circulating blood. The accelerating action upon coagulation of tissues or parts of tissues depends, as above stated, upon their content of kinase; but it also in part depends upon the fact that the tissue fluids excite the secretory activity of the form-elements.

FULD[1] has arrived at about the same results independently of MORAWITZ, but he has selected other names. The three substances thrombogen, kinase, and thrombin are called by him *plasmozym*, *cytozym*, and *holozym*. The chief reason why circulating blood remains fluid is, according to FULD, because the cytozym is only slowly formed therein and the ferment (holozym) produced thereby is in an inactive form. Another reason is that the blood contains an antibody for the fibrin ferment. The assumption of the presence of an antibody, generally antithrombin, in the circulating blood, which retards coagulation, does not only seem to be probable, but recently PUGLIESE[2] has isolated an antithrombin from blood and tissues.

A serum poor in ferment and having a weak action can be reactivated by the addition of acid or alkali (ALEX. SCHMIDT, MORAWITZ), and in this action, according to MORAWITZ, a thrombin (β) is produced which is somewhat different from α-thrombin. The β-thrombin is produced from a special β-prothrombin which never occurs in the plasma, but only in the serum. FULD explains this by the statement that the α-thrombin is changed in the serum into *metazym* (β-prothrombin), which is then transformed by the alkali or acid into *neozym* ($=\beta$-thrombin).

L. LOEB,[3] who has also conducted extensive investigations on the coagulation of the blood, ascribes, like other investigators, a great importance to the bodies existing in the tissue, which accelerate coagulation, and to which he gives the name *tissue coagulins*. These coagulins are indeed not identical with the coagulins of the blood-clot or the blood-serum, but, like these, act directly upon fibrinogen. Under favorable conditions the combined action of blood and tissue coagulins may be greater than the sum of the individual actions. He explains this by stating that an activation takes place by means of a kinase; still, though this is possible, he has not proved it.

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[1] Centralbl. f. Physiol., **17**. See also Fuld and Spiro, Hofmeister's Beiträge, **5**.

[2] Journ. de Physiol., **7**.

[3] The work of Loeb may be found in Medical News, New York, 1903. Virchow's Arch., **176**, and Hofmeister's Beiträge, **5**.

The coagulins of the blood are, according to LOEB, different from the tissue coagulins. The first show no specific action, i.e., not between invertebrates and vertebrates. The tissue coagulins, on the contrary, have by their action upon the blood a certain specificity, at least in animals widely separated from one another.

Based upon recent investigations, a short summary of the coagulation of the blood would be as follows: In the circulating blood-plasma there occur fibrinogen, lime salts, and probably also prothrombin. On account of the continued destruction of small amounts of form-elements, probably small quantities of thrombin are formed, which is destroyed or made inactive by the simultaneous presence of antithrombin. The reason why the blood remains fluid in life lies in the lack of thrombin. Under the influence of foreign bodies or of chemical irritants within or outside of the body the form-elements of the blood are incited to an increased secretory activity, and from them (perhaps also from the leucocytes in ovipara or from the leucocytes but chiefly from the blood-plates in mammalia) an abundance of kinase passes into the plasma. By this (as well as by the action of tissue fluids outside of the body) the thrombogen is transformed into α-prothrombin, which is changed by the lime salts into thrombin (α-thrombin). The latter transforms the fibrinogen into fibrin.

The bodies accelerating coagulation, like the tissue extracts and the lime salts, act upon the formation of thrombin. The mode of action of gelatine, if it has any accelerating action at all, is not known. The bodies retarding coagulation may in certain cases act directly upon the blood, either, like the neutral salts, retarding the development of the thrombin, or, like the oxalate or fluoride, preventing the same; or like the hirudin,[1] which, as an antithrombin, makes the thrombin inactive; or like the cobra-poison, which acts like an antikinase. In other cases they may have an indirect action, for they may, like the proteoses and others, cause the body to produce special bodies which stand in close relation to intravascular coagulation.

Intravascular Coagulation. It has been shown by ALEX. SCHMIDT and his students, as also by WOOLDRIDGE, WRIGHT,[2] and others, that an intravascular coagulation may be brought about by the intravenous injection into the circulating blood of a large quantity of a thrombin solution, as also by the injection of leucocytes or tissue fibrinogen (impure nucleoproteid). Intravascular coagulation may be brought about also under other conditions, such as after the injection of snake-poison (Martin[1] and others) or certain of the proteid-like colloid substances, synthetically prepared according to Grimaux's method (Halliburton and Pickering[2]). If too little of the above-mentioned bodies be injected, then we observe only a marked retarding tendency in the coagulation of the blood. According to Wooldridge it can generally be maintained that after a short stage of accelerated coagulability, which may lead to a total or partial intravascular coagulation, a second stage of a diminished or even arrested coagulability of the blood follows. The first stage is designated (Wooldridge) as the *positive* and the other as the *negative phase* of coagulation. These statements have been confirmed by several investigators.

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[1] The action of hirudin is somewhat doubtful. See Schittenhelm and Bodong, l.c.

[2] A Study of the Intravascular Coagulation, etc., Proceed. of the Roy. Irish Acad. (3), 2. See also Wright, Lecture on Tissue or Cell Fibrinogen, The Lancet, 1892; and Wooldridge's Method of Producing Immunity, etc., Brit. Med. Journal, Sept., 1891.

There is no doubt that the positive phase is brought about by an abundant introduction of thrombin, or by a rapid and abundant formation of the same. The explanation of the production of the negative phase, which can easily be brought about by pepsin proteoses, by various bodies such as extracts of crabs' muscles and other organs, eel-serum, enzymes, bacterial toxines, snake-poisons, etc., has been attempted in different ways. The best studied is the action of proteoses, but no conclusive results have been obtained thus far. The assertion of Pick and Spiro that the action of the proteoses does not depend upon the proteoses themselves, but upon a contaminating substance, the *protozym*, has been shown to be incorrect by Underhill. The bodies retarding coagulation obtained by Conradi[3] in autolysis, which are probably antithrombins, seem to act in a different way from the proteoses, and cannot for the present be made use of in explaining this question.

There are a large number of researches on the action of proteoses and of other retarding substances by different investigators, such as Grosjean, Ledoux, Contejean, Dastre, Floresco, Athanasiu, Carwallo, Gley, Pachon, Spiro and Ellinger, Fuld and Spiro, Morawitz and Nolf, but those of Delezenne[4] are of the greatest importance. We can say with certainty that the action is indirect and that the liver, and perhaps also the leucocytes and vessel walls (Nolf), are important for the process. The reasons for the non-coagulability of "peptone blood" are of two kinds: first, this blood contains an antithrombin, and, secondly, the thrombin for inexplicable reasons is absent, although such blood seems to contain thrombogen as well as kinase. The reason for the insufficient formation of thrombin is unknown, and only a few observations have been collected on the formation of antithrombin. According to NOLF, the peptone (more correctly the proteoses) causes an alteration in the leucocytes and the walls of the vessels, and a substance is secreted which brings about in the liver the formation of antithrombin. According to DELEZENNE, the proteoses bring about a destruction of leucocytes, and thereby a substance accelerating coagulation and another having a retarding action are set free. The first is destroyed by the liver, and hence the action of the retarding substance (the antithrombin) is obtained. The only thing that is positively proven is the part taken by the liver in this retardation of coagulation, as shown by GLEY and PACHON; the non-appearance of the thrombin formation is not explained by the above theories.

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[1] Journ. of Physiol., **15**.

[2] *Ibid.*, 18.

[3] Pick and Spiro, Zeitschr. f. physiol. Chem., **31**; Conradi, Hofmeister's Beiträge, **1**. See also Underhill, Amer. Journ. of Physiol., **9**.

[4] Grosjean, Travaux du laboratoire de L Fredericq, **4**, Liège, 1892; Ledoux, *ibid.*, **5**, 1896; Nolf, Bull. l'Acad. roy. de Belgique, 1902 and 1905, and Biochem. Centralbl., **3**; Spiro and Ellinger, Zeitschr. f. physiol. Chem., **23**; Fuld and Spiro, l. c.; Morawitz, l. c. The works of the above-mentioned French investigators can be found in Compt. rend. soc. biol., **46**, **47**, **48**, **50**, and **51**, and Arch. d. Physiol. (5), **7**, **8**, **9**, and **10**; see also especially Delezenne, Arch. d. Physiol. (5), **10**; Compt. rend. soc. biol., **51**, and Compt. rend., **130**.

The coagulation of the blood of lower animals may be of two kinds, according to L. LOEB.[1] A partial agglutination of the blood-cells may take place, and this kind of coagulation is the only kind in certain animals; but a true coagulation of fibrinogen may also take place. This latter coagulation is essentially the same as occurs in vertebrates, and here also an action of kinase (coagulin) upon thrombogen takes place.

The non-coagulability of cadaver blood depends usually, according to MORAWITZ,[2] upon the fact that it contains no fibrinogen, due to a fibrinolysis.

The *gases of the blood* will be treated of in Chapter XVII (on respiration).

IV. The Quantitative Composition of the Blood.

The quantitative analyses of the blood are of little value. We must ascertain on one side the relationship of the plasma and blood-corpuscles to each other, and on the other side the constitution of each of these two chief constituents. The difficulties which stand in the way of such a task, especially in regard to the living, non-coagulated blood, have not been removed. Since the constitution of the blood may differ not only in different vascular regions, but also in the same region under different circumstances, which renders also a number of blood analyses necessary, it can hardly appear remarkable that our knowledge of the constitution of the blood is still relatively limited.

The relative volume of blood-corpuscles and serum in defibrinated blood may be determined, according to L. and M. BLEIBTREU,[3] by various methods if the defibrinated blood is mixed with different proportions of isotonic NaCl solution (1 vol. of the blood to at least 1 vol. salt solution), the blood-corpuscles allowed to settle to the bottom, which may be facilitated by centrifugal force, and the clear supernatant mixture of serum and salt solution siphoned off. The methods are as follows:

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[1] Hofmeister's Beiträge, 5 and 6, and Virchow's Arch., 176. See also Ducceschi, Hofmeister's Beiträge, 3.

[2] Hofmeister's Beiträge, 8.

[3] Pflüger's Arch., 51, 55, and 60.

1. Determine the quantity of nitrogen in at least two different portions of the mixture of serum and salt solution by means of KJELDAHL'S method and calculate the quantity of protein corresponding thereto by multiplying with 6.25: and the relative volume of blood x, and also the volume of the structural elements $(1-x)$, are found by the following equation:

$$(e_1-e_2)x=\frac{s_2}{b_2}e_2-\frac{s_1}{b_1}e_1.$$

In this equation (for mixtures 1 and 2) b_1 or b_2 represents the volume of blood in the mixture, s_1 or s_2 the volume of salt solution, and e_1 or e_2 the quantity of protein in a certain volume of each mixture.

2. Determine the specific gravity of the blood-serum, of the salt solutions, and of at least one of the mixtures of serum and salt solution by means of a pycnometer. The relative volume of serum x is found in this by the following equation:

$$x=\frac{s}{b}\cdot\frac{S-K}{S_0-K}.$$

In this equation s and b represent the volumes of salt solution and blood mixed. S represents the specific gravity of the serum and salt solution obtained on allowing the blood-corpuscles to settle, S_0 the specific gravity of the serum, and K that of the salt solution.

For horse's blood two other shorter methods may be made use of (see the original article).

Important objections have been presented by several investigators, such as EYKMAN, BIERNACKI, and HEDIN,[1] against the above methods, whose value, therefore, is questionable. The same is also true for another method, suggested by ST. BUGARSKY and TANGL and partly corrected, in regard to the calculations, by STEWART.[2] This method is based upon a difference in the electrical conductivities of the blood and the plasma. According to the investigations of P. FRÄNCKEL,[3] the results obtained by determining the conductivities give the same figures as those by BLEIBTREU'S method, at least for human, horse, ox, and dog bloods. STEWART has also worked out a colorimetric method for the estimation of the volume of the blood-corpuscles and the plasma, which seems to be worth applying.

For clinical purposes the relative volume of corpuscles in the blood may be determined by the use of a small centrifuge called a *hæmatocrit*, constructed by BLIX and described and tested by HEDIN. A measured quantity of blood is mixed with a known volume (best an equal volume) of a fluid which prevents coagulation. This mixture is introduced into a tube and then centrifuged. According to HEDIN it is best to treat the blood, which is kept fluid by 1 p. m. oxalate, with an equal volume of a 9 p. m. NaCl solution. After complete centrifugalization, the layer of blood-corpuscles is read off on the graduated tube and the volume of blood-corpuscles (or more correctly the layer of blood-corpuscles) in 100 vols. of the blood calculated therefrom. By means of comparative counts, HEDIN and DALAND have found that an approximately constant relation exists between the volume of the layer of blood-corpuscles and the number of red corpuscles under physiological conditions, so that the number of corpuscles may be calculated from the volume. DALAND[1] has shown that such a calculation gives approximate results also in disease, when the size of the blood-corpuscles does not essentially deviate from the normal. In certain diseases, such as pernicious anæmia, this method gives such inaccurate results that it cannot be used.

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[1] Biernacki, Zeitschr. f. physiol. Chem., **19**; Eykman, Pflüger's Arch., **60**; Hedin, *ibid.*, and Skand. Arch. f. Physiol., **5**.

[2] Bugarsky and Tangl, Centralbl. f. Physiol., **11**; Stewart, Journ. of Physiol., **24**.

[3] Fränckel, Zeitschr. f. klin. Med., **52**.

KÖPPE[2] has recently shown that in centrifuging blood very rapidly, more than 5000 times per minute, the blood-corpuscles may be so completely separated that all intermediate fluid is removed. Because of the absence of this intermediate fluid the refraction is changed; the outer layers of the erythrocytes containing fat become transparent, and the column of blood-corpuscles becomes transparent and laky. If the volume of the separated column of blood-corpuscles is determined and the number of red blood-corpuscles counted, the absolute volume of these latter can be determined by this method.

In determining the relationship between the weight of blood-corpuscles and the weight of blood-fluid, we generally proceed in the following manner:

If any substance is found in the blood which belongs exclusively to the plasma and does not occur in the blood-corpuscles, then the amount of plasma contained in the blood may be calculated if we determine the amount of this substance in 100 parts of the plasma or serum respectively on one side, and in 100 parts of the blood on the other. If we represent the amount of this substance in the plasma by p and that in the blood by b, then the amount of x in the plasma from 100 parts of blood is $x = \frac{100 \, . \, b}{p}$.

Such a substance, which occurs only in the plasma, is fibrin according to HOPPE-SEYLER, sodium according to BUNGE (in certain kinds of blood), and sugar according to OTTO.[3] The experimenters just named have tried to determine the amount of the plasma and blood-corpuscles, respectively, in different kinds of blood, starting from the above-mentioned substances.

Another method suggested by HOPPE-SEYLER is to determine the total amount of hæmoglobin and proteins in a portion of blood, and on the other hand the amount of hæmoglobin and proteins in the blood-corpuscles (from an equal portion of the same blood) which have been sufficiently washed with common-salt solution by centrifugal force. The figure obtained as a difference between these two determinations corresponds to the amount of proteins which was contained in the serum of the first portion of blood. If we now determine the proteins in a special portion of serum of the same blood, then the amount of serum in the blood is easily determined. The usefulness of this method has been confirmed by BUNGE by the control experiments with the sodium determinations. If the amount of serum and blood-corpuscles in the blood is known, and we then determine the amount of the different blood-constituents in the blood-serum on one side and of the total blood on the other, the distribution of these different blood-constituents in the two chief components of the blood, plasma and blood-corpuscles, may be ascertained. In the table opposite are given analyses of the blood of various animals by ABDERHALDEN[1] according to HOPPE-SEYLER's and BUNGE's methods. The analyses of human blood by C. SCHMIDT[2] are older and were made according to another method, hence perhaps the results for the weights of the corpuscles are a little too high. All the results are in parts per 1000 parts of blood.

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[1] Hedin, Skand. Arch. f. Physiol., 2, 134 and 361, and 5; Pflüger's Arch., 60; Daland, Fortschritte d. Med., 9.

[2] Pflüger's Arch., 107.

[3] Hoppe-Seyler, Handb. d. physiol. u. path. chem. Analyse, 7. Aufl.; Bunge, Zeitschr. f. Biologie, 12; Otto, Pflüger's Arch., 35.

The relation between blood-corpuscles and plasma may vary considerably under different circumstances even in the same species of animal. In animals, in most cases considerably more plasma is found, sometimes two thirds of the weight of the blood.[3] For human blood ARRONET has found 478.8 p. m. blood-corpuscles and 521.2 p. m. serum (in defibrinated blood) as an average of nine determinations. SCHNEIDER[4] found 349.6 and 650.4 p. m. respectively in women.

The sugar occurs, it seems, only in the serum and not with the blood-corpuscles. The same is true, according to ABDERHALDEN, for the lime, fat, and perhaps also the fatty acids. The small traces of bile-acids found in normal blood are, according to CROFTAN,[5] contained in the leucocytes. The division of the alkalies between the blood-corpuscles and the plasma is different, as the blood-corpuscles from the pig, horse, and rabbit contain no soda, those from human blood are richer in potassium, and the corpuscles from ox-, sheep-, goat-, dog-, and cat-blood are considerably richer in sodium than potassium. Chlorine exists in all blood to a greater extent in the serum than in the blood-corpuscles. Iodine is found only in the serum, while iron occurs chiefly in the form-elements, especially in the erythrocytes. As the nucleoproteids contain iron, some iron always occurs in the leucocytes, and traces of iron are also found in the serum. This amount under normal conditions is very small, while in disease the relation between hæmoglobin-iron and other blood-iron does not seem to change

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[1] Zeitschr. f. physiol. Chem., **23** and **25**.

[2] Cited and in part recalculated from v. Gorup-Besanez, Lehrb. d. physiol. Chem., 4. Aufl., 345.

[3] See Sacharjin in Hoppe-Seyler's Physiol. Chem., 447; Otto, Pflüger's Arch., **35**; Bunge, l. c.; L. and M. Bleibtreu, Pflüger's Arch., **51**.

[4] Arronet, Maly's Jahresber., **17**; Schneider, Centralbl. f. Physiol., **5**, 362.

[5] Pflüger's Arch., **90**.

	Pig-blood.		Ox-blood.		Horse-blood.		Dog-blood.		Bull-blood.		Sheep-blood.	
	Blood-corpuscles, 435.09	Serum, 564.91	Blood-corpuscles, 325.5	Serum, 674.5	Blood-corpuscles, 397.7	Serum, 602.3	Blood-corpuscles, 442.8	Serum, 577.2	Blood-corpuscles, 334.3	Serum, 665.7	Blood-corpuscles. 319.2	Serum, 680.8
Water	272.20	518.36	192.65	616.25	243.87	551.14	277.71	514.30	206.81	608.03	200.39	624 16
Solids	162.89	46.54	132.85	58.249	153.84	51.15	165.10	42.89	127.50	57.66	118.82	56.63
Hæmoglobin	142.20	—	103.10	—	125.8	—	145.6	—	106.40	—	102.80	—
Proteid	8.35	38.26	20.89	48.901	20.05	42.65	2.36	34.05	15.38	46.41	12.80	46.56
Sugar	—	0.684	—	0.708	—	0.90	—	0.74	—	0.679	—	0.708
Cholesterin	0.213	0.231	1.100	0.835	0.26	0.31	0.56	0.37	0.610	0.599	1.147	0.891
Lecithin	1.504	0.805	1.220	1.129	1.93	1.05	1.02	0.98	0.953	1.244	1.329	1.088
Fat	—	1.104	—	0.625	—	0.50	—	0.91	—	2.357	—	0.859
Fatty acids	0.027	0.448	—	—	0.02	0.36	—	0.70	—	0.494	—	0.4908
Phosphoric acid as nuclein	0.0455	0.0123	0.0178	0.0089	0.05	0.01	0.05	0.01	0.0194	0.0089	0.0235	0.0109
Soda	—	2.401	0.7266	2.9084	—	2.62	1.27	2.39	0.839	2.873	0.760	2.917
Potash	2.157	0.152	0.2351	0.1719	1.32	0.15	0.11	0.14	0 233	0.174	0.236	0.172
Iron oxide	0.696	—	0.544	—	0.59	—	0.71	—	0.562	—	0.545	—
Lime	—	0.0689	—	0.0805	—	0.07	—	0.06	—	0.073	—	0.089
Magnesia	0.0656	0.0233	0.0056	0.0300	0.04	0.03	0.03	0.03	0.009	0.027	0.006	0.027
Chlorine	0.642	2.048	0.5901	2.4889	0.18	2.20	0.60	2.31	0.628	2.453	0.575	2.516
Phosphoric acid	0.8956	0.1114	0.2392	0.1646	0.98	0.15	0.67	0.14	0.236	0.156	0.228	0.163
Inorganic P_2O_5	0.7194	0.0296	0.1140	0.0571	0.76	0.05	0.54	0.05	0.133	0.041	0.088	0.057

	Goat-blood.		Cat-blood.		Rabbit-blood.		Human Blood, Man.		Human Blood, Woman.	
	Blood-corpuscles, 347 2	Serum, 652.8	Blood-corpuscles, 434.0	Serum, 566.0	Blood-corpuscles, 372.1	Serum, 627.9	Blood-corpuscles, 513.02	Serum, 486.98	Blood-corpuscles, 396.24	Serum, 603.76
Water	211.35	592.54	270.90	524.17	235.74	518.18	349.69	439.02	272 56	551.99
Solids	135.86	60.25	163.11	41.35	136 37	46.71	163.33	47.96	123.68	51.77
Hæmoglobin	112.50	—	143.2	—	123.50	—				
Proteid	18.76	50.96	11.62	33.16	4.55	33 63				
Sugar	—	0.822	—	0.860	—	1.036				
Cholesterin	0.601	0.698	0.556	0.339	0.268	0.343				
Lecithin	1.339	1.127	1.354	0.971	1.722	1.105	Organic bodies 159.59	43.82	120.13	46.70
Fat	—	0.0407	—	0.446	—	0.749				
Fatty acids	—	0.398	—	0.282	—	0.507				
Phosphoric acid as nuclein	0.028	0.0117	0.063	0.009	0.040	0.015	Inorg. 3.74	4.14	3.55	5.07
Soda	0.755	2.824	1.174	2 512	—	2.789	0.24	1.66	0.65	1.92
Potash	0.236	0.160	0.112	0.148	1 946	0.162	1.59	0.15	1.41	0.20
Iron oxide	0 547	—	0.694	—	0.615	—	—	—	—	—
Lime	—	0.078	—	0.062	—	0.072	—	—	—	—
Magnesia	0 014	0.026	0 035	0.024	0 029	0.028	—	—	—	—
Chlorine	0.514	2 409	0.455	2 360	0.460	2.438	0.90	1.72	0.36	0.14
Phosphoric acid	0.243	0.154	0.697	0.133	0.835	0.151	—	—	—	—
Inorganic P_2O_5	0.097	0.045	0.515	0.040	0.645	0.040	—	—	—	—

very much. There are also found in the blood manganese and traces of lithium, copper, lead, silver, and in menstrual blood arsenic has also been noted. The blood as a whole contains in ordinary cases 770–820 p. m. water, with 180–230 p. m. solids; of these 173–220 p. m. are organic and 6–10 p. m. inorganic. The organic consists, deducting 6–12 p. m. of extractive bodies, of proteins and hæmoglobin. The amount of this last-mentioned body in human blood is about 130–150 p. m. In the dog, cat, pig, and horse the quantity of hæmoglobin is about the same, but is lower in the blood from the ox, bull, sheep, goat, and rabbit (ABDERHALDEN).

The amount of sugar in the blood is on an average 1–1.5 p. m. It seems to be independent of the composition of the food, but feeding with large amounts of sugar or dextrin causes a considerable increase in the sugar of the blood, as observed by BLEILE. When the quantity of sugar amounts to more than 3 p. m., then, according to CL. BERNARD,[1] sugar occurs in the urine, and a glycosuria appears. An increase in the quantity of sugar takes place, as first observed by BERNARD and lately substantiated by FR. SCHENCK, after removal of blood. According to HENRIQUES[2] this increase of the reducing power, at least in dogs, is not due to sugar, but chiefly to jecorin, which substance is the cause of more of the reduction in normal blood than the sugar. It is difficult to judge of the value of many statements as to the amount of sugar and the reducing power of the blood, because the experimenters generally have not considered the presence of a certain quantity of jecorin or conjugated glucuronic acids, or they were unable to detect them.

The quantity of urea, which, according to SCHÖNDORFF, is equally divided between the blood-corpuscles and the plasma, is greater on taking food than in starvation (GRÉHANT and QUINQUAUD, SCHÖNDORFF) and varies between 0.2 and 1.5 p. m. In dogs SCHÖNDORFF found in starvation a minimum of 0.348 p. m. and a maximum of 1.529 p. m. at the point of highest urea formation. GOTTLIEB obtained much lower results by another direct method, namely, in starvation 0.1–0.2, and after meat feeding 0.28–0.56 p. m. In man v. JAKSCH[3] found 0.5–0.6 p. m. urea in normal blood. The quantity of urea is somewhat increased in fever, and in general in augmented protein metabolism and the increased urea formation depending thereon. A more important increase in the quantity of urea in the blood occurs in a retarded elimination of urea, as in cholera, also in cholera infantum and in infections of the kidneys and the urinary passages. After ligaturing the ureters or after extirpation of the kidneys of animals, an accumulation of urea takes place in the blood.

v. SCHRÖDER first showed that the blood of the shark was very rich in urea, and the quantity indeed amounted to 26 p. m. BAGLIONI[4] has recently shown that this large quantity of urea is of the greatest importance, as the presence of urea in these animals is a necessary life-condition for the heart and very probably for all organs and tissues.

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[1] Bleile, Arch. f. (Anat. u.) Physiol., 1879; Bernard, Leçons sur le diabète, Paris, 1877.

[2] Schenck, Pflüger's Arch., **57**; Henriques, Zeitschr. f. physiol. Chem., **23**. See also Kolisch and Stejskal, Wien. klin. Wochenschr., 1898.

[3] Gréhant et Quinquaud, Journ. de l'anatomie et de la physiol., **20**, and Compt. rend., **98**; Schöndorff, Pflüger's Arch., **54** and **63**; Gottlieb, Arch. f. exp. Path. u. Pharm., **42**; v. Jaksch, Leyden-Festschr., I, 1901.

[4] v. Schröder, Zeitschr. f. physiol. Chem., **14**; Baglioni, Centralbl. f. Physiol., **19**.

The blood also contains traces of ammonia. According to **Horodynski**, **Salaskin**, and **Zaleski**,[1] who worked with the improved **Nencki** and **Zaleski** method, the quantity in arterial dog-blood was 0.41 milligram in 100 grams of blood. The blood of the portal vein contains considerably more than the blood of the arteries, being 3–4.5 times richer; this is disputed by **Biedl** and **Winterberg**,[2] however. The blood from healthy persons contains on an average 0.90 milligram per 100 c.c., according to **Winterberg**.[3] The quantity of uric acid may be 0.1 p. m. in bird's blood (v. **Schröder**[4]). Uric acid has not been detected with positiveness in human blood under normal conditions, while it has been found in the blood in gout, croupous pneumonia, and certain other diseased conditions. Lactic acid was first found in human blood by **Salomon** and then by **Gaglio**, **Berlinerblau**, and **Irisawa**. The quantity of lactic acid may vary considerably. **Berlinerblau** found 0.71 p. m. as maximum. **Saito** and **Katsuyama**[5] found on an average 0.269 p. m. in hen's blood, and after carbon-monoxide poisoning the quantity increased to 1.227 p. m.

The Composition of the Blood in Different Vascular Regions and under Different Conditions.

Arterial and Venous Blood. The most striking difference between these two kinds of blood is the variation in color caused by their containing different amounts of gas and different amounts of oxyhæmoglobin and hæmoglobin. The arterial blood is light red; the venous blood is dark red, dichroitic, greenish by transmitted light through thin layers. The arterial coagulates more quickly than the venous blood. The latter, on account of the transudation which takes place in the capillaries, was formerly said to be somewhat poorer in water but richer in blood-corpuscles and hæmoglobin than the arterial blood; but this is denied by modern investigators. According to **Krüger**[6] and his pupils the quantity of dry residue and hæmoglobin in blood from the carotid artery and from the jugular vein (in cats) is the same. **Röhmann** and **Mühsam**[7] could not detect any difference in the quantity of fat in arterial and venous blood.

Blood from the Portal Vein and the Hepatic Vein. In consequence of the small quantities of bile and lymph found relatively to the large quantity of blood circulating through the liver in a given time, we can hardly expect to detect by chemical analysis a positive difference in the composition between the blood of the portal and hepatic veins. The statements in regard to such a difference are in fact contradictory. For example, DROSDOFF has found more hæmoglobin in the hepatic than in the portal vein, while OTTO found less. KRÜGER finds that the quantities of hæmoglobin, as well as of the solids, in the blood from the vessels passing to and from the liver are different, but a constant relationship cannot be determined. The disputed question as to the varying quantities of sugar in the portal and hepatic veins will be discussed in a following chapter (see Chapter VIII, on the formation of sugar in the liver). After a meal rich in carbohydrates, the blood of the portal vein not only becomes richer in dextrose, but may contain also dextrin and other carbohydrates (v. MERING, OTTO[1]). The amount of urea in the blood from the hepatic vein is greater than in other blood (GRÉHANT and QUINQUAUD[2]). In regard to the quantity of ammonia, see page 241.

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[1] Zeitschr. f. physiol. Chem., 35, which also gives the older literature.

[2] Pflüger's Arch., 88.

[3] Wien. klin. Wochenschr., 1897, and Zeitschr. f. klin. Med., 35.

[4] Ludwig's Festschrift, 1887.

[5] Irisawa, Zeitschr. f. physiol. Chem., 17, which also gives the older literature; Saito and Katsuyama, *ibid.*, 32.

[6] Zeitschr. f. Biologie, 26. This also gives the literature on the composition of the blood in different vascular regions.

[7] Pflüger's Archiv, 46.

Blood of the Splenic Vein is decidedly richer in leucocytes than the blood from the splenic artery. The red blood-corpuscles of the blood from the splenic vein are smaller than the ordinary, less flattened, and show a greater resistance to water. The blood from the splenic vein is also claimed to be richer in water, fibrin, and protein than the ordinary venous blood. According to v. MIDDENDORFF, it is richer in hæmoglobin than arterial blood. KRÜGER[3] and his pupils have found that the blood from the vena lienalis is generally richer in hæmoglobin and solids than arterial blood; still the contrary is often found. The blood from the splenic vein coagulates slowly.

The Blood from the Veins of the Glands. The blood circulates with greater rapidity through a gland during activity (secretion) than when at rest, and the outflowing venous blood has therefore during activity a lighter red color and a greater amount of oxygen. Because of the secretion the venous blood also becomes somewhat poorer in water and richer in solids.

The blood from the *Muscular Veins* shows an opposite behavior, for during activity it is darker and more venous in its properties because of the increased absorption of oxygen by the muscles and still greater production of carbon dioxide than when at rest.

Menstrual Blood, according to an old statement, has not the power of coagulating. This statement is nevertheless false, and the apparent uncoagulability depends in part on the retention of the blood-clot by the womb and the vagina, so that only fluid cruor is at times eliminated, and in part on a contamination with vaginal mucus, which disturbs the coagulation. Menstrual blood, according to GAUTIER and BOURCET, contains arsenic and is also richer in iodine than other blood (see blood-serum, page 187).

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[1] Drosdoff, Zeitschr. f. physiol. Chem., 1; Otto, Maly's Jahresber., 17; v. Mering, Arch. f. (Anat. u.) Physiol., 1877, 214.

[2] l. c.

[3] v. Middendorff, Centralbl. f. Physiol., 2, 753; Krüger, l. c.

The Blood of the Two Sexes. Woman's blood coagulates somewhat more quickly, has a lower specific gravity, a greater amount of water, and a smaller quantity of solids than the blood of man. The amount of blood-corpuscles and hæmoglobin is somewhat smaller in woman's blood. The amount of hæmoglobin is 146 p. m. for man's blood and 133 p. m. for woman's.

During *pregnancy* NASSE has observed a decrease in the specific gravity, with an increase in the amount of water, until the end of the eighth month. From then the specific gravity increases, and at delivery it is normal again. The amount of fibrin is somewhat increased (BECQUEREL and RODIER, NASSE). The number of blood-corpuscles seems to decrease. In regard to the amount of hæmoglobin the statements are somewhat contradictory. COHNSTEIN found the number of red corpuscles diminished in the blood of pregnant sheep as compared with non-pregnant, but the red corpuscles were larger and the quantity of hæmoglobin in the blood was greater in the first case. MÖLLENBERG[1] found in most cases an increase in the amount of hæmoglobin in pregnancy in the last months.

The Blood at Different Periods of Life. Fœtal and infant blood is richer in erythrocytes and hæmoglobin than the blood of the mother. The highest percentage of hæmoglobin in the blood has been observed by several investigators, such as COHNSTEIN and ZUNTZ, OTTO, WINTERNITZ, ABDERHALDEN, SCHWINGE, and others, immediately or very soon after birth or at least within the first few days. In man, two or three days after birth the hæmoglobin reaches a maximum (200–210 p. m.) which is greater than at any other period of life. This is the cause of the great abundance of solids in the blood of new-born infants, as observed by several investigators. The quantity of hæmoglobin and blood-corpuscles sinks gradually from this first maximum to a minimum of about 110 p. m. hæmoglobin, which minimum appears in human beings between the fourth and eighth years. The quantity of hæmoglobin then increases again until about the twentieth year, when a second maximum of 137–150 p. m. is reached. The hæmoglobin remains at this point only to about the forty-fifth year, and then gradually and slowly decreases (LEICHTENSTERN OTTO[2]). According to older statements, the blood at old age is poorer in blood-corpuscles and protein bodies, but richer in water and salts.

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[1] Nasse, Maly's Jahresber., 7; Becquerel and Rodier, Traité de chim. pathol., Paris, 1854; Cohnstein, Pflüger's Arch., 34, 233; Möllenberg, Maly's Jahresber., 31, 185. See also Payer, Arch. f. Gynäk., 71.

[2] Cohnstein and Zuntz, Pflüger's Arch., 34; Winternitz. Zeitschn. f. physiol. Chem., 22; Leichtenstern, Untersuch. über den Hämoglobingehalt des Blutes, etc., Leipzig,

The Influence of Food on the Blood. In complete starvation, no decrease in the amount of solid blood-constituents is found to take place (PANUM and others). The amount of hæmoglobin is increased a little, at least in the early period (SUBBOTIN, OTTO, HERMANN and GROLL, LUCIANI and BUFALINI), and also the number of red blood-corpuscles increases (WORM MÜLLER, BUNTZEN[1]), which probably depends partly on the fact that the blood-corpuscles are not so quickly transformed as the serum and partly on a greater concentration due to loss of water. In rabbits and to a less extent in dogs, POPEL found that complete abstinence had a tendency to increase the specific gravity of the blood. The amount of fat in the blood may be somewhat increased in starvation because the fat is taken up from the fat deposits and carried to the various organs by the blood (N. SCHULZ, DADDI[2]).

After a rich meal the relative number of blood-corpuscles, after secretion of digestive juices or absorption of nutritive liquids, may be increased or diminished (BUNTZEN, LEICHTENSTERN). The number of white blood-corpuscles may be considerably increased after a diet rich in proteins. After a diet rich in fat the plasma becomes, even after a short time, more or less milky-white, like an emulsion. The composition of the food acts essentially on the amount of hæmoglobin in the blood. The blood of herbivora is generally poorer in hæmoglobin than that of carnivora, and SUBBOTIN has observed in dogs after a partial feeding with food rich in carbohydrates that the amount of hæmoglobin sank from the physiological average of 137.5 p. m. to 103.2–93.7 p. m. TSUBOI[3] has also shown in experiments on rabbits and dogs that with an insufficient diet of bread and potatoes, where the body gave up protein and contained relatively much carbohydrate, the amount of hæmoglobin decreased and the blood became richer in water. According to LEICHTENSTERN, a gradual increase in the amount of hæmoglobin is found to take place in the blood of human beings on enriching the food, and according to the same investigator the blood of lean persons is generally somewhat richer in hæmo-

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1878; Otto, Maly's Jahresber., **15** and **17**; Abderhalden, Zeitschr. f. physiol. Chem., **34**; Schwinge, Pflüger's Arch., **73** (literature). See also Fehrsen, Journ. of Physiol., **30**.

[1] Panum, Virchow's Arch., **29**; Subbotin, Zeitschr. f. Biologie, **7**; Otto, l. c.; Worm Müller, Transfusion und Plethora, Christiania, 1875; Buntzen, see Maly's Jahresber., **9**; Hermann and Groll, Pflüger's Arch., **43**; Luciani and Bufalini, Maly's Jahresber., **12**.

[2] Popel, Arch. des scienc. biol. de St. Pétersbourg, **4**, 354; Schulz, Pflüger's Arch., **65**; Daddi, Maly's Jahresber., **30**.

[3] Subbotin, l. c.; Tsuboi, Zeitschr. f. Biologie, **44**.

globin than blood from fat ones of the same age. The addition of iron salts to the food greatly influences the number of blood-corpuscles and especially the amount of hæmoglobin they contain. The action of the iron salts is obscure.[1] There does not seem to be any doubt that not only is the iron contained in the food in the form of organic compounds active, but also iron salts and therapeutic iron. According to BUNGE and his pupils the iron preparations only act indirectly. They may combine with the sulphuretted hydrogen of the intestinal canal and thereby prevent the iron associated in the food as assimilable protein compounds from being eliminated as iron sulphide (BUNGE), or they may perhaps act as excitants upon the blood-forming organs (ABDERHALDEN).

An *increase in the number of red corpuscles*, a true "*plethora polycythæmia*," takes place after transfusion of blood of the same species of animal. According to the observations of PANUM and WORM MÜLLER,[2] the blood-liquid is quickly eliminated and transformed in this case—the water being eliminated principally by the kidneys and the protein burned into urea, etc.—while the blood-corpuscles are preserved longer and cause a "*polycythæmia*." A relative increase in the number of red corpuscles is found after abundant transudation from the blood, as in cholera and heart-failure with considerable congestion. An increase in the number of red blood-corpuscles has also been observed under the influence of diminished pressure or in high altitudes. VIAULT first called attention to the fact that the number of red corpuscles was very great in the blood of man and animals living in high regions. According to him the llama has about 16 million blood-corpuscles per cubic millimeter. By observations on himself and others, as well as on animals, VIAULT found the first effect of sojourning in high localities was a very considerable increase in the number of red corpuscles, in his own case 5–8 millions. A similar increase of the red blood-corpuscles, as also an increase in the quantity of hæmoglobin under the influence of diminished pressure, has been observed by many other investigators in human beings as well as in animals. Investigators are not united as to how this increase is brought about. The increase in the blood-corpuscles is not absolute but is only relative, and it is considered by several observers that there is neither a new formation nor a diminished destruction of the blood-corpuscles. A relative increase may be brought about in different ways. For example, another division of the blood-corpuscles in the vascular system has been supposed, whereby the blood-corpuscles accumulate in the capillaries, from which region the blood has been examined most often (Zuntz). It is also claimed that a concentration of the blood takes place by increased evaporation (Grawitz), and finally an increase in the blood-corpuscles has also been explained by assuming a contraction of the vascular system with the pressing out of plasma (Bunge, Abderhalden[1]). In connection with these experiments, it must be remarked that several trustworthy observations show that under the influence of diminished blood-pressure an actual increase in the red blood-corpuscles takes place, and Zuntz[2] and his co-workers have also shown that the activity in the red bone-marrow is increased.

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[1] See Bunge, Zeitschr. f. physiol. Chem., **9**; Häusermann, *ibid.*, **23**, where the works of Woltering, Gaule, Hall, Hochhaus, and Quincke are cited (the same work contains a table of the quantity of iron in various foods); Kunkel, Pflüger's Arch., **61**; Macallum, Journal of Physiol., **16**; Abderhalden, Zeitschr. f. Biologie, **39**.

[2] Panum, Virchow's Arch., **29**; Worm Müller, l. c.

A *decrease in the number of red corpuscles* occurs in anæmia from different causes. Every excessive hemorrhage causes an acute anæmia, or, more correctly, oligæmia. Even during the hemorrhage, the remaining blood becomes by diminished secretion and excretion, as also by an abundant absorption of parenchymous fluid, richer in water, somewhat poorer in proteins, and strikingly poorer in red blood-corpuscles. The oligæmia passes soon into a hydræmia. The amount of protein then gradually increases again; but the re-formation of the red blood-corpuscles is slower, and after the hydræmia follows also an oligocythæmia. After a little time the number of blood-corpuscles rises to normal; but the re-formation of hæmoglobin does not keep pace with the re-formation of the corpuscles, and a chlorotic condition may appear. A considerable decrease in the number of red corpuscles occurs also in chronic anæmia and chlorosis; still in such cases an essential decrease in the amount of hæmoglobin occurs without an essential decrease in the number of blood-corpuscles. The decrease in the amount of hæmoglobin is more characteristic of chlorosis than a decrease in the number of red corpuscles. The statements on the changes in the blood in anæmia and chlorosis differ very considerably, and in this connection attention must be called to the findings of Lorrain Smith (based on his estimation of the oxygen capacity and of the blood-volume) that in chlorosis an absolute diminution of the amount of hæmoglobin does not occur, but, that on the contrary, the total quantity of hæmoglobin may be normal, with only a relative diminution occurring, due to a pronounced increase of the blood-plasma and of the total quantity of blood.[3]

A very considerable decrease in the number of red corpuscles (300 000–400 000 in 1 c.mm.) and diminution in the amount of hæmoglobin $(\frac{1}{8}-{}^{1}\ )$ occurs in pernicious anæmia (Hayem, Laache, and others). On the contrary, the individual red corpuscles are larger and richer in hæmoglobin than they ordinarily are, and the number stands in an inverse relationship to the amount of hæmoglobin (Hayem). Besides this the red corpuscles often, but not always, show in pernicious anæmia remarkable and extraordinary irregularities of form and size, which Quincke[1] has termed *poikilocytosis.*

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[1] The literature on this subject may be found in Abderhalden, Zeitschr. f. Biologie, **43**; van Voornveld, Pflüger's Arch., **92**.

[2] Höhenklima und Bergwanderungen, by N. Zuntz, A. Loewy, Franz Müller, and W. Caspari, Berlin, 1906.

[3] Trans. Path. Soc. London, **51**, 1900. Complete analyses of chlorotic blood may be found in Erben, Zeitschr. f. klin. Med., **47**.

The *number of leucocytes* may, as stated above, be increased under physiological conditions as well as after a meal rich in protein (physiological leucocytosis). Under pathological conditions a high leucocytosis may occur, and this is especially found in leucæmia, which is characterized by a very great abundance of leucocytes in the blood. The number of leucocytes is markedly increased in this disease, and indeed not only absolutely, but also in relation to the number of red blood-corpuscles, which are increased to a considerable extent in leucæmia. Leucæmic blood has a lower specific gravity than the ordinary blood (1035–1040), and a paler color, as if it were mixed with pus. The reaction is alkaline, but after death it is frequently acid, probably due to a decomposition of lecithin, which is often considerably increased in leucæmia. Volatile fatty acids, lactic acid, glycerophosphoric acid, large amounts of xanthine bodies, and so-called Charcot's crystals (see semen, Chap. XIII) have also been found in leucæmic blood. The peptone (proteose) which is found in the leucæmic blood after death, and which does not exist in the fresh blood, is, according to Erben, a digestive product which is produced by a tryptic enzyme which originates from the leucocytes as well as by traces of a peptic enzyme. These enzymes, according to Erben, do not occur in normal blood, or are so firmly combined therein that on the death of the cells they are not set free, or at least their action does not become evident.[2]

A great number of investigations have been made on the chemical composition of blood in disease. But as we have only a few analyses of the blood of healthy individuals, and as the possible variations under physiological conditions are little known, it is difficult to draw any positive conclusions from the analyses of pathological blood. Unfortunately, on account of the large number of contradictory statements of the composition of the blood of diseased human beings, it is impossible to give a brief summary of the results, still the changes in the blood in disease must be of the greatest importance.

The *quantity of blood* is indeed somewhat variable in different species of animals and in different conditions of the body; in general we consider the entire quantity of blood in adults as about $\frac{1}{13}$ $\frac{1}{14}$ of the weight of the body, and in new-born infants about $\frac{1}{19}$. HALDANE and LORRAIN SMITH,[1] who have determined the quantity of blood by a new method, find in fourteen persons that it varies between $\frac{1}{16}$ and $\frac{1}{30}$ of the weight of the body. Fat individuals are relatively poorer in blood than lean ones. During inanition the quantity of blood decreases less quickly than the weight of the body (PANUM[2]), and it may therefore be also proportionally greater in starving individuals than in well-fed ones.

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[1] Laache, Die Anämie (Christiania, 1883), which also contains the literature; Quincke, Deutsch. Arch. f. klin. Med., 20 and 25. A complete chemical analysis of the blood has been made by Erben, Zeitschr. f. klin. Med., 40.

[2] Erben, Zeitschr. f. Heilkunde, 24, and Hofmeister's Beiträge, 5. See also Schumm, *ibid.*, 4 and 5.

By careful bleeding the quantity of blood may be considerably diminished without any dangerous symptoms. A loss of blood amounting to one fourth of the normal quantity has as a sequence no durable sinking of the blood-pressure in the arteries, because the smaller arteries accommodate themselves to the small quantities of blood by contracting (WORM MÜLLER[3]). A loss of blood amounting to one third of the quantity reduces the blood-pressure considerably, and a loss of one half of the blood in adults is dangerous to life. The more rapid the bleeding the more dangerous it is. New-born infants are very sensitive to loss of blood, and likewise fat, old, and weak persons cannot stand much loss of blood. Women can stand loss of blood better than men.

The quantity of blood may be considerably increased by the injection of blood from the same species of animal (PANUM, LANDOIS, WORM MÜLLER, PONFICK). According to WORM MÜLLER the normal quantity of blood may indeed be increased as much as 83 per cent without producing any abnormal conditions or lasting high blood-pressure. An increase of 150 per cent in the quantity of blood may, with a considerable variation in the blood-pressure, be directly dangerous to life (WORM MÜLLER). If the quantity of blood of an animal is increased by transfusion with blood of the same kind of animal, an abundant formation of lymph takes place. The water in excess is eliminated by the urine; and as the protein of the blood-serum is quickly decomposed, while the red blood-corpuscles are destroyed much more slowly (TSCHIRJEW, FORSTER, PANUM, WORM MÜLLER[4]), a polycythæmia is gradually produced.

The quantity of blood in the different organs depends essentially on their activity. During work the exchange of material in an organ is more pronounced than during rest, and the increased metabolism is connected with a more abundant flow of blood. Although the total quantity of blood in the body remains constant, the distribution of the blood in the various organs may be different at different times. As a rule the quantity of blood in an organ is an approximate measure of the more or less active metabolism going on in the same, and from this point of view the distribution of the blood in the different organs and groups of organs is of interest. According to RANKE,[1] to whom we are especially indebted for our knowledge of the relationship of the activity of the organs to the quantity of blood contained therein, of the total quantity of blood (in the rabbit) about one fourth comes to the muscles in rest, one fourth to the heart and the large blood-vessels, one fourth to the liver, and one fourth to the other organs.

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[1] Journ. of Physiol., **25**.

[2] Virchow's Arch., **29**.

[3] Transfusion und Plethora, Christiania, 1875.

[4] Panum, Nord. med. Ark., **7**; Virchow's Arch., **63**; Landois, Centralbl. f. d. med. Wissensch., 1875, and Die Transfusion des Blutes, Leipzig, 1875; Worm Müller, Transfusion und Plethora; Ponfick, Virchow's Arch., **62**; Tschirjew, Arbeiten aus der physiol. Anstalt zu Leipzig, 1874, 292; Forster, Zeitschr. f. Biologie, **11**; Panum, Virchow's Arch., **29**.

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[1] Die Blutvertheilung und der Thätigkeitswechsel der Organe, Leipzig, 1871.

CHAPTER VII.

CHYLE, LYMPH, TRANSUDATES AND EXUDATES.

I. Chyle and Lymph.

THE lymph is the mediator in the exchange of constituents between the blood and the tissues. The bodies necessary for the nutrition of the tissues pass from the blood into the lymph, and the tissues deliver water, salts, and products of metabolism to the lymph. The lymph, therefore, originates partly from the blood and partly from the tissues. From a purely theoretical standpoint one can, according to HEIDENHAIN, differentiate between blood-lymph and tissue-lymph according to origin. It is impossible at the present time to completely separate that which comes from the one or the other source. Chemically the lymph is the same as plasma and contains, at least to a great extent, the same bodies. The observation of ASHER and BARBÈRA,[1] that the lymph contains poisonous metabolic products, does not contradict such an assumption, as no doubt these products are transferred to the blood with the lymph. Although the blood does not show the same poisonous action as the lymph, still this can be explained by the great dilution these bodies undergo in the blood, and the difference between blood-plasma and lymph is no doubt of a quantitative nature. This difference consists chiefly in that the lymph is poorer in proteins. No essential chemical difference has been found between the lymph and the chyle of starving animals. After fatty food the chyle differs from the lymph in its wealth of minutely divided fat-globules, which give it a milky appearance; hence the old name "lacteal fluid."

Chyle and lymph, like the plasma, contain *seralbumin*, *serglobulins*, *fibrinogen*, and *fibrin ferment*. The two last-mentioned bodies occur only in very small amounts; therefore the chyle and lymph coagulate slowly (but spontaneously) and yield but little fibrin. Like other liquids poor in fibrin ferment, chyle and lymph do not at once coagulate completely, but repeated coagulations take place.

The extractive bodies seem to be the same as in plasma. *Sugar* (or at least a reducing substance) is found in about the same quantity as in the blood-serum, but in larger quantities than in the blood; this depends on the fact that the blood-corpuscles contain no sugar. The *glycogen* detected by DASTRE[1] in the lymph occurs only in the leucocytes. According to RÖHMANN and BIAL, lymph contains a diastatic enzyme similar to that in blood-plasma, and LÉPINE[2] has found that the chyle of a dog during digestion has great glycolytic activity. The amount of *urea* has been determined by WURTZ[3] as 0.12–0.28 p. m. The *mineral bodies* appear to be the same as in plasma.

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[1] Zeitschr. f. Biologie, 36.

As form-elements, *leucocytes* and *red blood-corpuscles* are common to both chyle and lymph. Chyle in fasting animals has the appearance of lymph. After fatty food it is, on the contrary, milky, due partly to small fat-globules, as in milk, and partly, indeed mostly, to finely divided fat. The nature of the *fat* occurring in chyle depends upon the kind of fat in the food. By far the greater part consists of neutral fat, and even after feeding with large quantities of free fatty acids, MUNK[4] found that the chyle contained chiefly neutral fat with only small amounts of fatty acids or soaps.

The *gases* of the chyle have not been studied, and it seems that the gases of an entirely normal human lymph have not thus far been investigated. The gases from dog-lymph contain only traces of oxygen and consist of 37.4–53.1 per cent CO_2 and 1.6 per cent N, calculated at 0° C., and 760 mm. mercury. The chief mass of the carbon dioxide of the lymph seems to be in firm chemical combination. Comparative analyses of blood and lymph have shown that the lymph contains more carbon dioxide than arterial, but less than venous blood. The tension of the carbon dioxide of lymph is, according to PFLÜGER and STRASSBURG,[5] smaller than in venous, but greater than in arterial blood.

The *quantitative composition of the chyle* must evidently be very variable.[6] The analyses thus far made refer only to that mixtureof chyle and lymph which is obtained from the thoracic duct. The specific gravity varies between 1.007 and 1.043. As an example of the composition of human chyle two analyses will be given. The first is by OWEN-REES, of the chyle of an executed person, and the second by HOPPE-SEYLER,[7] of the chyle in a case of rupture of the thoracic duct. In the latter case the fibrin had previously separated. The results are in 1000 parts.

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[1] Compt. rend. de soc. biol., **47**, and Compt. rend., **120**; Arch. de Physiol. (5), **7**.

[2] Röhmann and Bial, Pflüger's Arch., **52**, **53**, and **55**; Lépine, Compt. rend., **110**.

[3] Compt. rend., **49**.

[4] Virchow's Arch., **80** and **123**. In regard to the analysis of the fat of chyle, see Erben, Zeitschr. f. physiol. Chem., **30**.

[5] Hammarsten, Die Gase der Hundelymphe, Arbeiten aus d. physiol. Anstalt zu Leipzig, 1871; Strasburg, Pflüger's Archiv, **6**.

[6] See also Panzer, Zeitschr. f. physiol. Chem., **30**.

[7] Owen-Rees, cited from Hoppe-Seyler's Physiol. Chem., 595; Hoppe-Seyler, *ibid.*. 597. See also Carlier, Brit. Med. Journ., 1902, 175.

	No. 1.	No. 2.
Water	904.8	940.72 water
Solids	95.2	59.28 solids
Fibrin	Traces	....
Albumin	70.8	36.67 albumin
Fat	9.2	7.23 fat
Remaining organic bodies	10.8	2.35 soaps 0.83 lecithin 1.32 cholesterin 3.63 alcohol extractives 0.58 water extractives
Salts	4.4	6.80 soluble salts 0.35 insoluble salts

The quantity of fat is very variable and may be considerably increased by partaking of food rich in fats. I. Munk and A. Rosenstein[1] have investigated the lymph or chyle obtained from a lymph fistula at the end of the upper third of the leg of a girl eighteen years old and weighing 60 kg., and the highest quantity of fat in the chylous lymph was 47 p. m. after partaking of fat. In the starvation lymph from the same patient they found only 0.6–2.6 p. m. fat. The quantity of soaps was always small, and on partaking of 41 grams of fat the quantity of soaps was only about $\frac{1}{20}$ of the neutral fats.

A great many analyses of chyle from animals have been made, and they chiefly show the fact that the chyle is a liquid with a very changeable composition which stands closely related to blood-plasma, but with the chief difference that it contains more fat and less solids. The reader is referred to special works for these analyses, as, for example, to v. Gorup-Besanez's "Lehrbuch der physiologischen Chemie," 4th edition.

The *composition of the lymph* is also very changeable, and its specific gravity shows about the same variation as the chyle. In the following analyses, 1 and 2, made by Gubler and Quevenne, are the results obtained from lymph from the upper part of the thigh of a woman aged thirty-nine; and 3, made by v. Scherer, is an analysis of lymph from the sac-like dilated lymphatic vessels of the spermatic cord. No. 4 was made by C. Schmidt,[2] the data being obtained from lymph from the neck of a colt. The results are expressed in parts per 1000.

	1	2	3	4
Water	939.9	934.8	957.6	955.4
Solids	60.1	65.2	42.4	44.6
Fibrin	0.5	0.6	0.4	2.2
Albumin	42.7	42.8	34.7	....
Fat, cholesterin, lecithin	3.8	9.2	....	35.0
Extractive bodies	5.7	4.4	....	....
Salts	7.3	8.2	7.2	7.5

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[1] Virchow's Arch., **123**.

[2] Gubler and Quevenne, cited from Hoppe-Seyler's Physiol. Chem., 591; v. Scherer, *ibid.*, 591; C. Schmidt, *ibid.*, 592.

The salts found by C. SCHMIDT in the lymph of the horse have the following composition, calculated in parts per 1000 parts of the lymph:

Sodium chloride	5.67
Soda	1.27
Potash	0.16
Sulphuric acid	0.09
Phosphoric acid united with alkalies	0.02
Earthy phosphates	0.26

In the cases investigated by MUNK and ROSENSTEIN the quantity of solids in the fasting condition varied between 35.7 and 57.2 p. m. This variation was essentially dependent upon the extent of secretion, so that the low amount coincides with a more active secretion, and the reverse in the other case. The chief portion of the solids consisted of proteins, and the relationship between globulin and albumin was as 1:2.4 to 4. The mineral bodies in 1000 parts lymph (chylous) were: NaCl 5.83; Na_2CO_3 2.17; K_2HPO_4 0.28; $Ca_3(PO_4)_2$ 0.28; $Mg_3(PO_4)_2$ 0.09; and $Fe(PO_4)$ 0.025.

Under special conditions the lymph may be so rich in finely divided fat that it appears like chyle. Such lymph has been investigated by HENSEN in a case of lymph fistula in a ten-year-old boy, and by LANG[1] in a case of lymph fistula in the upper part of the left thigh of a girl of seventeen. The lymph investigated by HENSEN varied in the quantity of fat, as an average of nineteen analyses, between 2.8 and 36.9 p. m., while that investigated by LANG contained 24.85 p. m. of fat.

The quantity of lymph secreted must naturally change considerably under various conditions, and there are no means of measuring it. The size of the flow of lymph is, as HEIDENHAIN suggests, no measure of the abundance of supply of nutritive material to the organs, and the lymph-tubes act according to him as "drain-tubes," removing the excess of fluid from the lymph-fissures as soon as the pressure therein rises to a certain height. Attempts have been made to determine the quantity of lymph flowing in 24 hours in the thoracic duct of animals. According to HEIDENHAIN the quantity averages 640 c.c. for a dog weighing 10 kilos.

Determinations of the quantity of lymph in man have also been attempted. NOËL-PATON[2] obtained 1 c.c. of lymph per minute from the severed thoracic duct of a patient weighing 60 kilos. The quantity in the 24 hours cannot be calculated from this amount. In the case of MUNK and ROSENSTEIN, 1134–1372 grams chyle was collected within 12–13 hours after partaking of food. In the fasting condition or after starving for 18 hours they found 50 to 70 grams per hour, sometimes 120 grams and above, especially in the first few hours after powerful muscular exercise.

Several circumstances have a marked influence on the extent of lymph secretion. During starvation less lymph is secreted than after partaking of food. NASSE[1] has observed in dogs that the formation of lymph is increased 36 per cent more after feeding with meat than after feeding with potatoes, and about 54 per cent more than after 24 hours' deprivation of food. In this connection mention must be made of the important observations of ASHER and BARBÈRA[2] that with pure protein diet the lymph current is increased in the thoracic cavity, and also that the increase in the lymph secretion runs parallel with the elimination of nitrogen in the urine, i.e., with the absorption of the protein from the digestive tract.

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[1] Hensen, Pflüger's Arch., **10**; Lang, see Maly's Jahresber., **4**.

[2] Journ. of Physiol., **11**.

An increase in the total quantity of blood, as by transfusion of blood; also especially on preventing the flow of blood by means of ligatures, causes an increase in the quantity of lymph. According to HEIDENHAIN, on the contrary, a very considerable change in the pressure in the aorta causes only a little change in the abundance of the lymph-flow. The quantity of lymph may be raised by powerfully active and passive movements of the limbs (LESSER). Under the influence of curare, an increase of the lymph secretion is observed (PASCHUTIN, LESSER[3]), and the quantity of solids in the lymph is also increased.

The bodies inciting lymph-flow, the so-called *lymphagogues*, are of especially great interest, and they may, according to HEIDENHAIN,[4] be divided into two different chief groups. The lymphagogues of the first series—extracts of crab-muscles, blood-leech, anodons, liver and intestine of dogs, as well as peptone and egg albumin, strawberry extracts, metabolic products of bacteria and others—cause a greatly increased secretion of lymph without raising the blood-pressure, and in this way the blood-plasma becomes poorer in proteins and the lymph richer than before. For the formation of this lymph, which HEIDENHAIN designates blood-lymph, we must admit with him that a special secretory activity of the capillary-wall endothelium exists. The lymphagogues of the second series, such as sugar, urea, sodium chloride, and other salts, also cause an abundant lymph formation. The blood, as well as the lymph, thereby becomes richer in water. This increased amount of water depends, according to HEIDENHAIN, upon an increased delivery of water by the tissue-elements, and this lymph is chiefly tissue-lymph, according to him. Diffusion is no doubt of great importance in the formation of this lymph, but the secretory activity of the endothelium is also of importance, at least for certain bodies, such as sugar.

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[1] Cited from Hoppe-Seyler, Physiol. Chem., 593.

[2] The works of Asher and collaborators, Barbèra, Gies, and Busch, upon lymph formation may be found in Zeitschr. f. Biologie, **36**, **37**, **40**.

[3] Lesser, Arbeiten aus der physiol. Anstalt zu Leipzig, Jahrgang **6**; Paschutin, *ibid.*, **7**.

[4] Heidenhain, Pflüger's Arch., **49**; Hamburger, Zeitschr. f. Biologie, **27** and **30**. See especially Ziegler's Beitr. zur Path. u. zur allg. Pathol., **14**, 443; also Arch. f. (Anat. u.) Physiol., 1895 and 1896.

In the past, the formation of lymph was explained in a purely physical way by the united action of filtration from the blood and the osmosis between the blood and tissue-fluid. Later HEIDENHAIN and HAMBURGER ascribed a special activity to the capillary endothelium, assuming that they take part in the formation of lymph in a secretory manner.

Another view which also besides the physical processes is of especial physiological moment in the explanation of lymph formation was suggested by ASHER and his collaborators (BARBÈRA, GIES, and BUSCH). According to them the lymph is a product of the work of the organs; its amount is dependent upon an increased or diminished activity of the organs, and the lymph is therefore a measure of the work in these. The close relation between lymph formation and the work of organs has also been shown for several of them, especially for the liver. STARLING has shown that after the introduction of lymphagogues of the first series, chiefly liver lymph is secreted, which he claims is a proof against HEIDENHAIN'S view, and he explains the increased permeability of the vessel wall by the fact that these bodies have a poisonous irritating action. On the contrary, ASHER explains this increased lymph-flow by the statement that the substance in question—as well as those influences which incite the activity of the liver—produces an increased formation of lymph in these organs. This view is supported by experiments upon the action of lymphagogues on blood coagulation and liver activity (DELEZENNE and others), for, according to GLEY, these bodies have at the same time a lymphagogue action and an action upon the secretion of the glands. We have no direct evidence of the action of the lymphagogues of the first series upon the organs, but we know from KUSMINE'S work that peptone, leech extract, and the extractives of the crab-muscles act directly upon the liver-cells and bring about morphological changes. The connection between organ activity and lymph formation has also been shown upon muscles and glands by others besides the above-mentioned investigators (HAMBURGER, BAINBRIDGE [1]).

The extent of organ work certainly essentially influences the quantity and properties of the lymph. Still from this we cannot draw any positive conclusions as to whether the lymph formation is brought about by physicochemical processes alone or whether in this process a specific, not closely definable secretory force is at work at the same time. In regard to this much-disputed question attention must be called in the first place to the fact that the important works of HEIDENHAIN, HAMBURGER, LAZARUS-BARLOW, and others, as well as the investigations of ASHER and GIES and of MENDEL and HOOKER [2] upon the lengthy post-mortem lymph-flow, have shown that the older filtration hypothesis is untenable. That the part played by filtration as compared with that of the osmotic force is only very trivial has been conclusively shown by the adherents of the physico-chemical theory of lymph formation.

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[1] In regard to the works cited, as well as the literature upon lymph formation, see Ellinger, "Die Bildung der Lymphe," Ergebnisse der Physiol., I, Abt. 1, 1902, and Asher, Biochem. Centralbl., 4.

[2] Amer. Journ. of Physiol., 7.

Several investigators (KORANYI, STARLING, ROTH, ASHER, and others) have shown clearly that the work in the glands and tissue-cells must cause a difference in the osmotic pressure upon the two sides of the capillary wall. That this is so follows from several circumstances and especially from the fact that, in dissimilation in the cells, bodies of high molecular weight are split into a number of smaller molecules, which latter, either directly, if they leave the cells and pass into the tissue-fluid, or indirectly, when they remain in the cells, produce an increase in the osmotic tension within the cells, and in this way cause a taking up of water from the fluid and must therefore increase the osmotic pressure of the tissue-fluids. As the cells can by synthesis build up highly complex constituents from simple molecules, and as the chief products of catabolism are carbon dioxide and water, it is difficult to explain these intricate conditions. Still, irrespective of whatever view, a change in one or the other direction in the osmotic pressure upon both sides of the capillary wall must be produced hereby. Whether this and other physico-chemical processes are alone sufficient to explain the lymph formation (COHNSTEIN, ELLINGER) remains an open and disputed question.[1]

II. Transudates and Exudates.

The serous membranes are normally kept moistened by liquids whose quantity is sufficient only in a few instances, as in the pericardial cavity and the subarachnoidal space, for a complete chemical analysis to be made of them. Under diseased conditions an abundant transudation may take place from the blood into the serous cavities, into the subcutaneous tissues, or under the epidermis; and in this way pathological transudates are formed. Such true transudates, which are similar to lymph, are generally poor in form-elements and leucocytes, and yield only very little or almost no fibrin, while the inflammatory transudates, the so-called exudates, are generally rich in leucocytes and yield proportionally more fibrin. As a rule, the richer a transudate is in leucocytes the closer it stands to pus, while a diminished quantity of leucocytes renders it more nearly like a real transudate or lymph.

It is ordinarily accepted that filtration is of the greatest importance in the formation of transudates and exudates. The facts coincide with this view that all these fluids contain the salts and extractive bodies occurring in the blood-plasma in about the same quantity as the blood-plasma, while the amount of proteins is habitually smaller. While the different fluids belonging to this group have about the same quantities of salts and extractive bodies, they differ from one another chiefly in containing differing quantities of protein and form-elements, as well as varying quantities of transformation and decomposition products of these latter—changed blood-coloring matters, cholesterin, etc. The correspondence in the amount of salts and extractive bodies present in the blood and in transudates supplies just as little proof for a filtration as it does for the formation of lymph; but still it cannot be doubted for other reasons that filtration is often of great importance in the formation of a transudate. To what extent filtration is active in the perfectly normal vascular wall cannot be answered.

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[1] On this question see Ellinger, "Die Bildung der Lymphe," Ergebnisse der Physiologie, I, Abt. 1, 355, and Asher, Biochem. Centralbl., 4, pp. 1 and 45.

The altered permeability of the capillary walls in disease is a second important factor in the formation of transudates. The circumstance that the greatest quantity of protein occurs in transudates in inflammatory processes, to which is also due the abundant quantity of form-elements in such transudates, has been explained by this hypothesis. The greater quantity of protein in the transudates in formative irritation is in great part explained by the large amount of destroyed form-elements. The interesting observation made by PAIJKULL,[1] that in such cases in which an inflammatory irritation has taken place the fluid contains nucleoalbumin (or nucleoproteid?), while this substance does not occur in transudates in the absence of inflammatory processes, can be explained by the presence of form-elements. Still, such a phosphorized protein substance does not occur in all inflammatory exudates.

As the secretory importance of the capillary endothelium has been made probable by the investigations of HEIDENHAIN, it is *a priori* to be expected that an abnormally increased secretory activity of the endothelium is a cause of transudates. Those observations which substantiate such an assumption can also be explained just as well by assuming a changed permeability of the capillary walls.

The varying quantities of protein observed by C. SCHMIDT[2] in the tissue-fluids in different vascular regions can perhaps be explained by the different condition of the capillary endothelium. For example, the amount of protein in the PERICARDIAL, PLEURAL, and PERITONEAL FLUIDS is considerably greater than in those fluids which are found in the SUBARACHNOIDAL SPACE, in the SUBCUTANEOUS TISSUES, or in the AQUEOUS HUMOR, which are poor in protein. The condition of the blood also greatly affects the transudates, for in hydræmia the amount of protein in the transudate is very small. With the increase in the age of a transudate, of a hydrocele fluid for instance, the quantity of protein is increased, probably by resorption of water, and indeed exceptional cases may occur in which the amount of protein, without any previous hemorrhage, is even greater than in the blood-serum.

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[1] See Maly's Jahresber., 22.

[2] Cited from Hoppe-Seyler, Physiol. Chem., 607

The proteins of transudates are chiefly seralbumin, serglobulin, and a little fibrinogen. Proteoses and peptones do not occur, excepting perhaps in the cerebrospinal fluid, and in those cases where an autolysis has taken place in the liquid.[1] The non-inflammatory transudates as a rule undergo spontaneous coagulation not at all, or only very slowly. On the addition of blood or blood-serum they coagulate. Inflammatory exudates coagulate spontaneously, and PAIJKULL has shown that these often contain nucleoproteid (or nucleoalbumin). In inflammatory exudates a protein substance has been habitually observed which is precipitated by acetic acid, but which does not occur in transudates, or only in very small quantities. This substance, which has been observed and studied by MORITZ, STAEHELIN, UMBER, and RIVALTA, is claimed by the first three observers to be free from phosphorus, while RIVALTA considers it to be a phosphorized pseudoglobulin. UMBER calls it *serosamucin*, although it yields only very little reducing carbohydrate. According to JOACHIM[2] it is only a part of the globulin, a view which cannot be correct for all cases. v. HOLST[3] has so far substantiated UMBER's observation in that he has isolated a mucin substance from an ascitic fluid in carcinoma of the stomach and the peritoneum, which seemed to be identical with UMBER's serosamucin, as well as with the synovial mucin. There does not seem to be any doubt that in transudates and exudates different protein substances may occur under different circumstances, although the globulins form besides seralbumin ordinarily the chief mass of the protein bodies. Mucoid substances, which were first observed by HAMMARSTEN in certain cases of ascites without complications with ovarial tumors, and which are cleavage products of a more complicated substance, seem according to PAIJKULL[4] to be regular constituents of transudates and are closely related to the above-mentioned serosamucin.

There are numerous investigations on the relationship between globulin and seralbumin, and JOACHIM has recently determined the relationship between euglobulin and the total globulin. No conclusive results can be drawn from these determinations. The relationship between globulin and seralbumin varies very much in different cases, but, as Hoffmann and Pigeand[1] have shown, the variation is in each case the same as in the blood-serum of the individual.

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[1] Umber, Münch. med. Wochenschr., 1902, and Berlin. klin. Wochenschr., 1903. In regard to the autolysis in transudates, see also Galdi, Biochem. Centralbl., **3**; Eppinger, Zeitschr. f. Heilkunde, **35**; and Zak, Wien. klin. Wochenschr., 1905.

[2] Paijkull, l. c.; Moritz, Münch. med. Wochenschr., 1903; Staehelin, *ibid.*, 1902; Umber, Zeitschr. f. klin. Med., **48**; Rivalta, Biochem. Centralbl., **2** and **5**; Joachim, Pflüger's Arch., **93**.

[3] Zeitschr. f. physiol. Chem., **43**.

[4] Hammarsten, *ibid.*, **15**; Paijkull, l. c.

The specific gravity runs nearly parallel with the quantity of protein. The varying specific gravity has been suggested as a means of differentiation between transudates and exudates by Reuss,[2] as the first often show a specific gravity below 1015–1010, while the others have a specific gravity of 1018 or above. This rule holds good in many, but not in all cases.

The *gases* of the transudates consist of carbon dioxide besides small amounts of nitrogen and traces of oxygen. The tension of the carbon dioxide is greater in the transudates than in the blood. When mixed with pus, the amount of carbon dioxide is decreased.

The *extractives* are, as above stated, the same as in the blood-plasma; but sometimes extractive bodies occur, such as *allantoin* in dropsical fluids (Moscatelli[3]), which have not been detected in the blood. *Urea* seems to occur in very variable amounts. *Sugar* also occurs in transudates, but it is not known to what extent the reducing power is due, as in blood-serum, to other bodies. A reducing, non-fermentable substance has been found by Pickardt in transudates. The sugar is generally dextrose, but levulose seems to have been found[4] in several cases. *Sarcolactic acid* has been found by C. Külz[5] in the pericardial fluid from oxen. *Succinic acid* has been found in a few cases in hydrocele fluids, while in other cases it is entirely absent. *Leucine* and *tyrosine* have been found in transudates from diseased livers and in pus-like transudates which have undergone decomposition, and after autolysis. Among other extractives found in transudates must be mentioned *uric acid, xanthine, creatine, inosite,* and *pyrocatechin* (?).

The division of the nitrogenous substances in human transudates and exudates has so far been little studied. Otori[6] has found that no essential difference exists between serous exudates and transudates in regard to the quantity of urea and amino-acids. The amount of total nitrogen and proteins runs parallel with the specific gravity, and the same is generally true for the absolute values for amino-acid nitrogen and purine nitrogen.

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[1] Joachim, l. c.; Hoffmann, Arch. f. exp. Path. u. Pharm., 16; Pigeand, see Maly's Jahresber., 16.

Reuss, Deutsch. Arch. f. klin. Med., 28. See also Otto, Zeitschr. f. Heilkunde, 17.

[3] Zeitschr. f. physiol. Chem., 13.

[4] Pickardt, Berl. klin. Wochenschr., 1897. See also Rotmann, Münch. med. Wochenschr., 1898; Neuberg and Strauss, Zeitschr. f. physiol. Chem., 36.

[5] Zeitschr. f. Biologie, 32.

[6] Zeitschr. f. Heilkunde, 25.

The amino-acid nitrogen and the urea nitrogen in pus are greater as the specific gravity rises. In serous exudates and transudates, on the contrary, the amino-acid nitrogen and the urea nitrogen are not proportional to the specific gravity, but are dependent upon the general circulatory condition of the body.

The investigations upon the molecular concentration have shown that no essential and constant difference exists between exudates and transudates. The osmotic concentration and the concentration of the electrolytes are as a rule the same as in blood-serum, although sometimes rather divergent results have been found. The concentration of the electrolytes shows according to Bodon,[1] like the blood-serum, much less variation than the total concentration. The alkalinity determined by titration is about the same in transudates and exudates and is equal to that of the blood-serum. The determination of the HO-ion concentration has shown that the transudates and exudates in this regard are about as neutral as the blood-serum (Bodon).

As above stated, irrespective of the varying number of form-elements contained in the different transudates, the quantity of protein is the most characteristic chemical distinction in the composition of the various transudates; therefore a quantitative analysis is of importance only in so far as it considers the quantity of protein. On this account, in the following, relative to the quantitative composition, chief stress will be put on the quantity of protein.

Pericardial Fluid. The quantity of this fluid is, even under physiological conditions, so large that a sufficient quantity for chemical investigation has been obtained (from persons who had been executed). This fluid is lemon-yellow in color, somewhat sticky, and yields more *fibrin* than other transudates. The amount of solids, according to the analyses performed by v. Gorup-Besanez, Wachsmuth, and Hoppe-Seyler,[2] is 37.5–44.9 p. m., and the amount of protein is 22.8–24.7 p. m. The analysis made by Hammarsten of a fresh pericardial fluid from a young man who had been executed yielded the following results, calculated in 1000 parts by weight:

Water	960.85		
Solids	39.15		
Proteins	28.60	Fibrin	0.31
		Globulin	5.95
		Albumin	22.34
Soluble salts	8.60	NaCl	7.28
Insoluble salts	0.15		
Extractive bodies	2.00		

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[1] Pflüger's Arch., **104**, where the literature on this subject may be found.

[2] v. Gorup-Besanez, Lehrbuch d. physiol. Chem., 4. Aufl., 401; Wachsmuth, Virchow's Arch., **7**; Hoppe-Seyler, Physiol. Chem., 605.

Friend[1] has found nearly the same composition for a pericardial fluid from a horse, with the exception that this liquid was relatively richer in globulin. The ordinary statement that pericardial fluids are richer in fibrinogen than other transudates is hardly based on sufficient proof. In a case of chylopericardium, which was probably due to the rupture of a chylous vessel or caused by a capillary exudation of chyle because of stoppage, **Hasebroek**[2] found in 1000 parts of the fluid 103.61 parts solids, 73.79 parts proteins, 10.77 parts fat, 3.34 parts cholesterin, 1.77 parts lecithin, and 9.34 parts salts.

The **pleural fluid** occurs under physiological conditions in such small quantities that a chemical analysis of it cannot be made. Under pathological conditions this fluid may show very variable properties. In certain cases it is nearly serous, in others again sero-fibrinous, and in others similar to pus. There is a corresponding variation in the specific gravity and the properties in general. If a pus-like exudate is kept enclosed for a long time in the pleural cavity, a more or less complete maceration and solution of the pus-corpuscles is found to take place. The ejected yellowish-brown or greenish fluid may then be as rich in solids as the blood-serum; and an abundant flocculent precipitate of a nucleoalbumin or nucleoproteid (the *pyin* of early writers) may be obtained on the addition of acetic acid. This precipitate is soluble with difficulty in an excess of acetic acid.

Numerous analyses, by many investigators,[3] of the quantitative composition of pleural fluids under pathological conditions have been published. From these analyses we learn that in hydrothorax the specific gravity is lower and the quantity of protein less than in pleuritis. In the first case the specific gravity is generally less than 1.015, and the quantity of protein 10–30 p. m. In acute pleuritis the specific gravity is generally higher than 1020, and the quantity of protein 30–65 p. m. The quantity of fibrinogen, which in hydrothorax is about 0.1 p. m., may amount to more than 1 p. m. in pleuritis. In pleurisy with an abundant accumulation of pus, the specific gravity may rise even to 1.030, according to the observations of HAMMARSTEN. The quantity of solids is often 60–70 p. m., and may be even more than 90–100 p. m. (HAMMARSTEN). Mucoid substances have also been detected in pleural fluids by PAIJKULL. Cases of chylous pleurisy are also known; in such a case MÉHU[4] found 17.93 p. m. fat and cholesterin in the fluid.

The quantity of **peritoneal fluid** is very small under physiological conditions. The investigations refer only to the fluid under diseased conditions (*dropsical* or *ascitic fluid*). The color, transparency, and consistency of these may vary greatly.

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[1] Halliburton, Text-book of Chem. Physiol., etc., London, 1904.

[2] Zeitschr. f. physiol. Chem., 12.

[3] See the works of Méhu, Runeberg, F. Hoffmann, Reuss, all of which are cited in Bernheim's paper in Virchow's Arch., **131**, 274. See also Paijkull, l. c., and Halliburton's Text-book, 346; Joachim, l. c.

[4] Arch. gén. de méd., 1886, 2, cited from Maly's Jahresber.. **16**.

In cachectic conditions or a hydræmic condition of the blood the fluid has little color, is milky, opalescent, watery, does not coagulate spontaneously, has a very low specific gravity, 1.005–1.010–1.015. and is nearly free from form-elements. The ascitic fluid in portal stagnation, or in general venous congestion, has a low specific gravity and ordinarily less than 20 p. m. protein, although in certain cases the quantity of protein may rise to 35 p. m. In carcinomatous peritonitis it may have a cloudy, dirty-gray appearance, due to its richness in form-elements of various kinds. The specific gravity is then higher, the quantity of solids greater, and it often coagulates spontaneously. In inflammatory processes it is straw- or lemon-yellow in color, somewhat cloudy or reddish, due to leucocytes and red blood-corpuscles, and from great richness in leucocytes it may appear more like pus. It coagulates spontaneously and may be relatively richer in solids. It contains regularly 30 p. m. or more protein (although exceptions with less protein occur), and may have a specific gravity of 1.030 or above. On account of the rupture of a chylous vessel, the dropsical fluid may be rich in very finely emulsified fat (CHYLOUS ASCITES). In such cases 3.86–10.30 p. m. fat has been found in the dropsical fluid (GUINOCHET, HAY[1]), and even 17–43 p. m. has been found by MINKOWSKI.

As first shown by GROSS, an ascitic fluid may have a chylous appearance without the presence of fat, i.e., pseudochylous. The cause of the chylous properties of a transudate is not known, although numerous investigators, such as GROSS, BERNERT, MOSSE, and STRAUSS, have studied the subject; several observations, however, seem to show that it is connected with the amount of lecithin contained therein. In a case investigated by H. WOLFF[2] the oleic-acid ester of cholesterin was combined either chemically or molecularly with the euglobulin.

By admixture of ascitic fluid with the fluid from an ovarian cyst the former may sometimes contain pseudomucin (see Chapter XIII). There are also cases in which the ascitic fluid contains mucoids which may be precipitated by alcohol after removal of the proteins by coagulation at boiling temperature. Such mucoids, which yield a reducing substance on boiling with acids, have been found by HAMMARSTEN in tuberculous peritonitis and in cirrhosis hepatis syphilitica in men. According to the investigations of PAIJKULL, these substances seem to occur often and perhaps habitually in the ascitic fluids.

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[1] Guinochet, see Strauss, Arch. de Physiol., 18. See Maly's Jahresber., **16**, 475.

[2] Gross, Arch. f. exp. Path. u. Pharm., **44**; Bernert, *ibid.*, **49**; Mosse, Leyden's Festschrift, 1901; Strauss, cited in Biochem. Centralbl., **1**, 437; Wolff, Hofmeister's Beiträge, **5**.

As the quantity of protein in ascitic fluids is dependent upon the same factors as in other transudates and exudates, it is sufficient to give the following example of the composition, taken from BERNHEIM's[1] treatise. The results are expressed in 1000 parts of the fluid:

	Max.	Min.	Mean.
Cirrhosis of the liver	34.5	5.6	9.69—21.06
Bright's disease	16.11	10.10	5.6 —10.36
Tuberculous and idiopathic peritonitis	55.8	18.72	30.7 —37.95
Carcinomatous peritonitis	54.20	27.00	35.1 —58.96

JOACHIM found the highest relative globulin amounts and lowest albumin percentages in cirrhosis; in carcinoma, on the contrary, the lowest globulin and the highest albumin. The values in cardiac stagnation stand between the cirrhosis and carcinoma percentages.

Urea has also been found in ascitic fluids, sometimes only as traces, sometimes in larger quantities (4 p. m. in albuminuria), also *uric acid, allantoin* in cirrhosis of the liver (MOSCATELLI), *xanthine, creatine, cholesterin, sugar, diastatic* and *proteolytic enzymes*, and according to HAMBURGER[2] also a *lipase.*

Hydrocele and Spermatocele Fluids. These fluids differ essentially from each other in various ways. The hydrocele fluids are generally colored light or dark yellow, sometimes brownish with a shade of green. They have a relatively higher specific gravity, 1.016–1.026, with a variable but generally higher amount of solids, an average of 60 p. m. They sometimes coagulate spontaneously, sometimes only after the addition of fibrin ferment or blood. They contain *leucocytes* as chief form-elements. Sometimes they contain smaller or larger amounts of *cholesterin crystals.*

The spermatocele fluids, on the contrary, are as a rule colorless, thin, and cloudy like water mixed with milk. They sometimes have an acid reaction. They have a lower specific gravity, 1.006–1.010, a lower amount of solids—an average of about 13 p. m.—and do not coagulate either spontaneously or after the addition of blood. They are, as a rule, poor in protein and contain *spermatozoa, cell-detritus,* and *fat-globules* as form-constituents. To show the unequal composition of these two kinds of fluids we will give the average results (calculated in parts per 1000 parts of the fluid) of seventeen analyses of hydrocele fluids and four of spermatocele fluids made by HAMMARSTEN.[3]

	Hydrocele.	Spermatocele.
Water	938.85	986.83
Solids	61.15	12.17
Fibrin	0.59	.....
Globulin	13.25	0.59
Seralbumin	35.94	1.82
Ether extractive bodies	4.02	10.76
Soluble salts	8.60	
Insoluble salts	0.66	

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[1] l. c. As it was impossible to derive mean figures from those given by Bernheim, the author has given the maximum and minimum of the averages given by him.

[2] Arch. f. (Anat. u.) Physiol., 1900, 433.

[3] Upsala Läkaref. Förh., 14, and Maly's Jahresber., 8, 347.

In the hydrocele fluid traces of *urea* and a reducing substance have been found, and in a few cases also *succinic acid* and *inosite*. A hydrocele fluid may, according to DEVILLARD,[1] sometimes contain paralbumin or metalbumin (?). Cases of chylous hydrocele are also known.

Cerebrospinal Fluid. The cerebrospinal fluid is thin, water-clear, of low specific gravity, 1.007–1.008. The spina bifida fluid is very poor in solids, 8–10 p. m., with only 0.19–1.6 p. m. protein. The fluid of chronic hydrocephalus is somewhat richer in solids (13–19 p. m.) and proteins. According to HALLIBURTON the protein of the cerebrospinal fluid is a mixture of *globulin* and *proteose;* occasionally some peptone occurs, and more rarely, in special cases, seralbumin appears. The statements of HALLIBURTON on the occurrence of *proteose* do not coincide with the observations of other investigators (PANZER, SALKOWSKI[2]). In general paralysis HALLIBURTON and MOTT have obtained a *nucleoproteid* in the cerebrospinal fluid. *Choline* occurs in several diseases, as in general paralysis, brain-tumors, tabes dorsalis, and epilepsy (HALLIBURTON and MOTT, DONATH[3]). *Dextrose,* or at least a fermentable sugar, occurs habitually in the cerebrospinal fluid, while the statements of HALLIBURTON as to the occurrence of a substance similar to pyrocatechin could not be substantiated by NAWRATZKI,[4] and hence this substance does not exist in all cerebrospinal fluids. *Urea* occurs in cerebrospinal fluids, but not always. The variable relationship between potassium and sodium[5] is probably due, according to SALKOWSKI, to the absence or presence of fever during the formation of the exudate; the amount of potassium is high in the acute cases and low in the chronic ones. According to CAVAZZANI,[6] who has especially studied the cerebrospinal fluids, the alkalinity of these fluids is considerably less than that of the blood and independent of this last fluid. For this and several other reasons CAVAZZANI draws the conclusion that the cerebrospinal fluid is formed by a true secretory process.

Aqueous Humor. This fluid is clear, alkaline towards litmus, and has a specific gravity of 1.003–1.009. The amount of solids is on an average 13 p. m., and the amount of proteins only 0.8–1.02 p. m. The protein consists of *seralbumin* and *globulin* and very little *fibrinogen.* According to GRUENHAGEN it contains *paralactic acid,* another dextrogyrate substance, and a *reducing body* which is not similar to dextrose or dextrin. Pautz[1] found *urea* and *sugar* in the aqueous humor of oxen.

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[1] Bull. Soc. chim., **49**, 617.

[2] Halliburton's Text-book; Panzer, Wien. klin. Wochenschr., 1899; Salkowski, Jaffé Festschrift, 265.

[3] Halliburton and Mott, Phil. Transact. Roy. Soc. London, Series B, **191**; Donath, Zeitschr. f. physiol. Chem., **39** and **42**; see also Mansfield, *ibid.*, **42.**

[4] Zeitschr. f. physiol. Chem., **23.** See also Rossi, *ibid.*, **39** (literature).

[5] See Salkowski, l. c. New quantitative analyses of cerebrospinal and hydrocephalus fluids may be found in the cited works of Nawratzki, Panzer, and Salkowski.

[6] See Maly's Jahresber., **22**, 346, and Centralbl. f. Physiol., **15**, 216.

Blister-fluid. The content of blisters caused by burns, and of vesicatory blisters and the blisters of the *pemphigus chronicus*, is generally a fluid rich in solids and proteins (40–65 p. m.). This is especially true of the contents of vesicatory blisters. In a burn-blister K. Mörner[2] found 50.31 p. m. proteins, among which were 13.59 p. m. globulin and 0.11 p. m. fibrin. The fluid contains a substance which reduces copper oxide, but no pyrocatechin. The fluid of the pemphigus is alkaline in reaction. A wound secretion collected by Lieblein[3] under aseptic conditions was alkaline in reaction and contained less protein than the blood-serum. It formed a slight fibrin clot and contained proteoses only at first or at the beginning of the abscess formation. As the wound healed, the relationship between the globulin and albumin changed, and on the third day of the healing the quantity of albumin was at least nine tenths of the total protein.

The fluid of **subcutaneous œdema.** This is, as a rule, very poor in solids, purely serous, does not contain fibrinogen, and has a specific gravity of 1.005–1.013. The quantity of proteins is in most cases lower than 10 p. m.,—according to Hoffmann 1–8 p. m.,—and in serious affections of the kidneys, generally with amyloid degeneration, less than 1 p. m. has been shown (Hoffmann[4]). The œdematous fluid also habitually contains *urea*, 1–2 p. m., and *sugar*.

The FLUID OF THE ECHINOCOCCUS cyst is related to the transudates and is poor in proteins. It is thin and colorless, and has a specific gravity of 1.005–1.015. The quantity of solids is 14–20 p. m. The chemical constituents are *sugar* (2.5 p. m.), *inosite*, traces of *urea*, *creatine*, *succinic acid*, and salts (8.3–9.7 p. m.). Proteins are found only in traces, and then only after an inflammatory irritation. In the last-mentioned case 7 p. m. proteins have been found in the fluid.

The Synovial Fluid and Fluid in Synovial Cavities around Joints, etc. The synovia is hardly a transudate, but it is often discussed in an appendix to the transudates.

The synovia is an alkaline, sticky, fibrous, yellowish fluid which is cloudy, from the presence of cell-nuclei and the remains of destroyed cells, but is also sometimes clear. It contains also, besides *proteins* and salts, a mucin substance, *synoviamucin* (v. Holst[5]). In pathological synovia Hammarsten has found a mucin-like substance which is not mucin. It behaves like a nucleoalbumin or a nucleoproteid and gives no reducing substance on boiling with acids. Salkowski[6] also found a mucin-like substance in a pathological synovial fluid, which was neither mucin nor nucleoalbumin. He called the substance *synovin*.

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[1] Gruenhagen, Pflüger's Arch., **43**; Pautz, Zeitschr. f. Biologie, **31**.

[2] Skand. Arch. f. Physiol., **5**.

[3] Habilitationsschrift Prag, 1902, printed by H. Laupp, Tübingen.

[4] Deutsch. Arch. f. klin. Med., **44**.

[5] Zeitschr. f. physiol. Chem., **43**.

[6] Hammarsten, Maly's Jahresber., **12**; Salkowski, Virchow's Arch., **131**.

The composition of synovia is not constant, but is different in rest and in motion. In the last-mentioned case the quantity of fluid is less, but the amount of the mucin-like body, of proteins, and of the extractive bodies is greater, while the quantity of salts is diminished. This may be seen from the following analyses by Frerichs.[1] The figures represent parts per 1000.

	I. Synovia from a Stall-fed ox.	II. Synovia from a Field-fed ox.
Water	969.9	948.5
Solids	30.1	51.5
Mucin-like body	2.4	5.6
Albumin and extractives	15.7	35.1
Fat	0.6	0.7
Salts	11.3	9.9

The synovia of new-born babes corresponds to that of resting animals. The fluid of the bursæ mucosæ, as also the fluid in the synovial cavities around joints, etc., is similar to synovia from a qualitative standpoint.

III. Pus.

Pus is a yellowish-gray or yellowish-green, creamy mass of a faint odor and an unsavory, sweetish taste. It consists of a fluid, the *pus-serum*, in which solid particles, the *pus-cells*, swim. The number of these cells varies so considerably that the pus may at one time be thin and at another time so thick that it scarcely contains a drop of serum. The specific gravity, therefore, may also greatly vary, namely, between 1.020 and 1.040, but ordinarily it is 1.031–1.033. The reaction of fresh pus is generally alkaline, but it may become neutral or acid from a decomposition in which fatty acids, glycerophosphoric acid, and also lactic acid are formed. It may become strongly alkaline when putrefaction occurs with the formation of ammonia.

In the chemical investigation of pus, the pus-serum and the pus-corpuscles must be studied separately.

Pus-serum. Pus does not coagulate spontaneously nor after the addition of defibrinated blood. The fluid in which the pus-corpuscles are suspended is not to be compared with the blood-plasma, but rather with the serum. The pus-serum is pale yellow, yellowish green, or brownish yellow, and has an alkaline reaction towards litmus. It contains, for the most part, the same constituents as the blood-serum; but sometimes besides these—when, for instance, the pus has remained in the body for a long time—it contains a nucleoalbumin or a nucleoproteid which is precipitated by acetic acid and is soluble with great difficulty in an excess of the acid (*pyin* of the older authors). This nucleoalbumin seems to be formed from the hyaline substance of the pus-cells by maceration. The pus-serum contains, moreover, at least in many cases, no fibrin ferment. According to the analyses of Hoppe-Seyler[1] the pus-serum contains in 1000 parts:

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[1] Wagner's Handwörterbuch, 3, Abt., 41 63.

	I.	II.
Water	913.70	905.65
Solids	86.30	94.35
Proteins	63.23	77.21
Lecithin	1.50	0.56
Fat	0.26	0.29
Cholesterin	0.53	0.87
Alcohol extractives	1.52	0.73
Water extractives	11.53	6.92
Inorganic salts	7.73	7.77

The ash of pus-serum has the following composition, calculated to 1000 parts of the serum:

	I.	II.
$NaCl$	5.22	5.39
Na_2SO_4	0.40	0.31
Na_2HPO_4	0.98	0.46
Na_2CO_3	0.49	1.13
$Ca_3(PO_4)_2$	0.49	0.31
$Mg_3(PO_4)_2$	0.19	0.12
PO_4 (in excess)	....	0.05

The **pus-corpuscles** are generally thought to consist in great part of emigrated white blood-corpuscles, and their chemical properties have therefore been given in discussing these. The molecular granules, fat-globules, and red blood-corpuscles are considered rather as casual form-elements.

The pus-cells may be separated from the serum by centrifugal force, or by decantation directly or after dilution with a solution of sodium sulphate in water (1 vol. saturated sodium-sulphate solution and 9 vols. water), and then washed by this same solution in the same manner as the blood-corpuscles.

The chief constituents of the pus-corpuscles are proteins of which the largest portion seems to be a nucleoproteid which is insoluble in water and which expands into a tough, slimy mass when treated with a 10 per cent common-salt solution. This protein substance, which is soluble in alkali but is quickly changed thereby, is called Rovida's *hyaline substance*, and the property of the pus of being converted into a slime-like mass by a solution of common salt depends on this substance. Besides this substance the pus-cells contain also a *globulin* which coagulates at 48–49° C., as well as *serglobulin* (?), *seralbumin*, a substance similar to coagulated protein (Miescher), and lastly *peptone* or proteose (Hofmeister[2]). It is very remarkable that no nucleohistone or histone has been detected in the pus-cells.

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[1] Med.-chem. Untersuch., 490.

[2] Miescher in Hoppe-Seyler's Med.-chem. Untersuch., 441; Hofmeister, Zeitschr. f. physiol. Chem., 4.

There are also found in the protoplasm of the pus-cells, besides the proteins, *lecithin, cholesterin, xanthine bodies, fat,* and *soaps.* HOPPE-SEYLER has found *cerebrin,* a decomposition product of a protagon-like substance, in pus (see Chapter XII). KOSSEL and FREYTAG[1] have isolated from pus two substances, *pyosin* and *pyogenin,* which belong to the cerebrin group (see Chapter XII). HOPPE-SEYLER[2] claims that *glycogen* appears only in the living, contractile white blood-cells and not in the dead pus-corpuscles. Several other investigators have nevertheless found glycogen in pus. The cell-nucleus contains *nuclein* and *nucleoproteids.* MANDEL and LEVENE[3] have shown the occurrence of *glucothionic acid* in the pus-cells.

In regard to the occurrence of *enzymes* in the pus-cells it must be remarked that neither thrombin nor prothrombin is found therein, although these bodies are generally considered as being derived from the leucocytes and can also be obtained from the thymus leucocytes. The occurrence in the pus-cells, besides catalases and oxidases, of a proteolytic enzyme is of great interest. It is not only important for the intracellular digestion and for the amount of proteoses in the pus-cell, but also for the solution of the fibrin clot and pneumonic infiltrations (FR. MÜLLER, O. SIMON[4]).

The *mineral constituents* of the pus-corpuscles are potassium, sodium, calcium, magnesium, and iron. A part of the alkalies exists as chlorides, and the remainder, as well as the chief part of the other bases, exists as phosphates.

The quantitative composition of the pus-cells from the analyses of HOPPE-SEYLER is as follows, in parts per 1000 of the dried substance:

	I.		II.
Proteins	137.62	685.85	673.69
Nuclein	342.57		
Insoluble bodies	205.66		
Lecithin	143.83		75.64
Fat			75.00
Cholesterin	74.00		72.83
Cerebrin	51.99		102.84
Extractive bodies	44.33		

MINERAL SUBSTANCES IN 1000 PARTS OF THE DRIED SUBSTANCE.

NaCl	4.34
$Ca_3(PO_4)_2$	2.05
$Mg_3(PO_4)_2$	1.13
$FePO_4$	1.06
PO_4	9.16
Na	0.68
K	Traces (?)

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[1] Zeitschr. f. physiol. Chem., **17**, 452.

[2] Hoppe-Seyler, Physiol. Chem., 790.

[3] Biochem. Zeitschrift. 4.

[4] Fr. Müller, Verhandl. Nat. Gesellsch. zu Basel, 1901; O. Simon, Deutsch. Arch. f. klin. Med., 70.

Miescher has obtained other results for the alkali compounds, namely, potassium phosphate 12, sodium phosphate 6.1, earthy phosphate and iron phosphate 4.2, sodium chloride 1.4, and phosphoric acid combined with organic substances 3.14–2.03 p. m.

In pus from congested abscesses which have stagnated for some time occur *peptone* (proteose), *leucine* and *tyrosine*, free *fatty acids* and *volatile fatty acids*, such as formic acid, butyric acid and valerianic acid. There are also found *chondrin* (?) and *glutin* (?), *urea*, *dextrose* (in diabetes), *bile-pigments* and *bile-acids* ((in catarrhal icterus).

As more specific but not constant constituents of the pus must be mentioned the following: *pyin*, which seems to be a nucleoproteid precipitable by acetic acid, and also *pyinic acid* and *chlorrhodinic acid*, which have been so little studied that they cannot be more fully treated here.

In many cases a blue, more rarely a green, color has been observed in the pus. This depends on the presence of micro-organisms (*Bacillus pyocyaneus*). From such pus Fordos and Lücke[1] have isolated a crystalline blue pigment, *pyocyanin*, and a yellow pigment, *pyoxanthose*, which is produced from the first by oxidation.

Appendix.

LYMPHATIC GLANDS, SPLEEN, ETC.

The Lymphatic Glands. The cells of the lymphatic glands are found to contain the protein substances occurring generally in cells (Chapter V, pages 141 and 142). According to Bang[2] they also contain histone nucleates (*nucleohistone*), but in smaller amounts and of a different variety from the better-studied nucleohistone from the thymus gland. Proteoses occur as products of autolysis. By a lengthy autolysis of lymph-glands Reh[3] found ammonia, tyrosine, leucine (somewhat scanty), thymine, and uracil among the cleavage products. Besides the other ordinary tissue constituents, such as collagen, reticulin, elastin, and nuclein, there occur in the lymphatic glands also *cholesterin*, *fat*, *glycogen*, *sarcolactic acid*, *xanthine bodies*, and *leucine*. In the inguinal glands of an old woman Oidtmann found 714.32 p. m. water, 284.5 p. m. organic and 1.16 p. m. inorganic substances. In the cells of the mesenteral lymphatic glands of oxen Bang[4] found 804.1 p. m. water, 195.9 p. m. solids, 137.9 total proteins, 6.9 p. m. histone nucleate 10.6 p. m. nucleoproteid, 47.6 p. m. bodies soluble in alcohol, and 10.5 p. m. mineral constituents.

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[1] Fordos, Compt. rend., 51 and 56; Lücke, Arch. f. klin. Chirurg., 3; Boland, Centralbl. f. Bakt. u. Parasit., I, 25.

[2] Studier over Nucleoproteider, Kristiania, 1902, and Hofmeister's Beiträge, 4.

[3] Hofmeister's Beiträge, 3.

[4] l. c.

The Thymus. The cells of this gland are very rich in nuclein bodies and relatively poor in the ordinary proteins, but their nature has not been closely studied. The chief interest is attached to the nuclein substances. KOSSEL and LILIENFELD first prepared from the watery extract of the gland, by precipitating with acetic acid and then further purifying, a protein substance which has been generally called *nucleohistone*. By the action of dilute hydrochloric acid upon nucleohistone it splits, according to these investigators, into histone and leuconuclein. The leuconuclein is a true nuclein; hence it is a nucleic-acid compound with protein which is relatively poor in protein and rich in phosphorus. The more recent investigations of BANG, MALENGREAU, and HUISKAMP[1] upon nucleohistone all show that this nucleoproteid is not a unit substance but a mixture of at least two bodies. The views of the investigators mentioned differ quite essentially from one another as to the nature of these bodies, but this is partly due to the different methods used by them and partly to the ready changeability of the substances in question.

Besides the real nucleohistone, B-nucleoalbumin of MALENGREAU, LILIENFELD's histone contains a second nucleoproteid which BANG and HUISKAMP call simple nucleoproteid, while MALENGREAU designates it A-nucleoalbumin. This protein, which contains only about 1 per cent phosphorus and which is possibly identical with the nucleoproteid found by LILIENFELD in the thymus, yields a nuclein, but no free nucleic acid, on cleavage. As second cleavage product it yields, according to MALENGREAU, the A-histone, which can be readily precipitated by magnesium and ammonium sulphates from the ordinary B-histone of the thymus gland. The occurrence of A-histone in the gland has been verified by BANG, and according to BANG and HUISKAMP the A-histone is not derived from the nucleoproteid, as these investigators claim that it yields no histone. According to BANG the nucleoproteid yields only an albuminate, besides the nuclein, as cleavage products.

The true nucleohistone, which is much richer in phosphorus (the calcium salt containing, according to BANG, on an average 5.23 per cent P), yields ordinary histone as one cleavage product and free nucleic acid as the other, according to the unanimous opinion of the above-mentioned investigators. According to BANG, whose statements on this point have been substantiated by MALENGREAU, it splits on saturating with NaCl into nucleic acid and histone without yielding any other protein. On this account BANG does not consider this body as nucleohistone in the ordinary sense, i.e., not as a nucleoproteid, but as a histone nucleate. The nucleohistone behaves like an acid, whose salts, especially the calcium salt, have been closely studied by HUIS-

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[1] Lilienfeld, Zeitschr. f. physiol. Chem., 18; Kossel, *ibid.*, **30** and **31**; Bang, *ibid.*, **30** and **31**. See also Arch. f. Math. og Naturvidenskab, **25**, Kristiania, 1902, and Hofmeister's Beiträge, **1** and **4**; Malengreau, La Cellule, **17** and **19**; Huiskamp, Zeitschr. f. physiol. Chem., **32**, **34**, and **39**.

KAMP. On the electrolysis of a solution of alkali nucleohistone in water HUISKAMP found also that the nucleohistone collected in traces at the anode, and that the sodium compound is therefore ionized in the solution. The nucleic acid-calcium-histone compound has been prepared, it seems, in a pure state by BANG, and he found the following average composition: C 43.69; H 5.60; N 16.87; S 0.47; P 5.23; Ca 1.71 per cent. The question as to what compound contains the A-histone remains to be investigated.

The nucleohistone prepared by HUISKAMP's method by precipitating with $CaCl_2$ is, according to him, a mixture of two nucleohistones, of which one, the α-nucleohistone, contains 4.5 per cent phosphorus, and the other, β-nucleohistone, contains, on the contrary, only in round numbers 3 per cent phosphorus.[1] As the two nucleohistones are poorer in phosphorus than the nucleic acid-histone compound analyzed by BANG, and as HUISKAMP on cleavage of his preparation did not, like BANG and MALENGREAU, obtain pure nucleic acid, it is still a question whether HUISKAMP was working with sufficiently pure substances.

In regard to the methods used by the above investigators in the isolation of the bodies in question we must refer to the original publications.

In connection with the so-called nucleohistone, attention must be called to *tissue fibrinogen* and *cell fibrinogen*, which are compound proteins, and are claimed by certain investigators to stand in close relation to the coagulation of the blood. These may be in part nucleoproteids and in part also nucleohistones. To this same group belong also the important cell constituents described by ALEX. SCHMIDT[2] and called *cytoglobin* and *preglobulin*. The cytoglobin, which is soluble in water, may be considered as the alkali compound of preglobulin. The residue of the cells left after complete extraction with alcohol, water, and salt solution has been called *cytin* by ALEX. SCHMIDT.

Besides the above-mentioned and the ordinary bodies belonging to the connective-tissue group, small quantities of *fat*, *leucine*, *succinic acid*, *lactic acid*, *sugar*, and traces of *iodothyrin* are present. According to GAUTIER[3] *arsenic* also occurs in very small amounts, and no doubt here as well as in other organs it is related to the nuclein substances. The richness in nuclein bodies explains the occurrence of large quantities of *purine bases*, chiefly *adenine*, whose quantity, according to KOSSEL and SCHINDLER,[4] is 1.79 p. m. in the fresh organ and 19.19 p. m. in the dry substance. The bodies *thymine* and *uracil* (?) obtained, besides lysine and ammonia, by KUTSCHER, as products of autodigestion of the gland, probably have a similar origin. LILIENFELD[5] has found *inosite* and *protagon* in the cells of the thymus. Among the enzymes, besides *arginase*, *guanase*, and *adenase*, we must especially mention the enzyme studied by JONES, which acts like a *nuclease*, splitting off phosphoric acid and purine bases from the nucleoproteids. This enzyme, contrary to trypsin, acts best in acid liquids and is readily destroyed by alkalies at body temperature.[1] The quantitative composition of the lymphocytes of the thymus of a calf is, according to LILIENFELD's analysis, as follows. The results are given in 1000 parts of the dried substance.

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[1] Zeitschr. f. physiol. Chem., **39**.

[2] See foot-note 5, p. 141.

[3] Compt. rend., **129**.

[4] Zeitschr. f. physiol. Chem., **13**.

[5] Kutscher, *ibid.*, **34**; Lilienfeld, *ibid.*, **18**.

Proteids	17.7
Leuconuclein	687.9
Histone	86.7
Lecithin	75.1
Fat	40.2
Cholesterin	44.0
Glycogen	8.0

The dried substance of the leucocytes amounted to an average of 114.9 p. m. Potassium and phosphoric acid are prominent mineral constituents. LILIENFELD found KH_2PO_4 amongst the bodies soluble in alcohol.

Attention must be called to the analyses of BANG,[2] which show that the thymus contains about the same quantity of nucleoproteid, but about five times as much histone nucleate as the lymphatic glands—calculated in both cases upon the same amount of dry substance. OIDTMANN[3] found 807.06 p. m. water, 192.74 p. m. organic and 0.2 p. m. inorganic substances in the gland of a child two weeks old.

The Spleen. The pulp of the spleen cannot be freed from blood. The mass which is separated from the spleen capsule and the structural tissue by pressure and which ordinarily serves as material for chemical investigations is therefore a mixture of blood and spleen constituents. For this reason the proteins of the spleen are little known. The nucleoproteid isolated by LEVENE and MANDEL is to be considered as a true spleen constituent. The *ferruginous albuminate* has been considered as a spleen constituent for a long time, and especially also a protein substance which does not coagulate on boiling, and which is precipitated by acetic acid and yields an ash containing much phosphoric acid and iron oxide.[4]

The pulp of the spleen, when fresh, has an alkaline reaction, but quickly turns acid, due partly to the formation of free *paralactic acid* and partly perhaps to *glycerophosphoric acid*. Besides these two acids there have been found in the spleen also *volatile fatty acids*, as formic, acetic, and butyric acids, as well as *succinic acid, neutral fats, cholesterin,* traces of *leucine, inosite* (in ox-spleen), *scyllite,* a body related to inosite (in the spleen of Plagiostoma), *glycogen* (in dog-spleen), *uric acid, xanthine bodies,* and *jecorin.* LEVENE has found in the spleen a *glucothionic acid,* i.e., an acid which is related to chondroitin-sulphuric acid but not identical therewith, and which gives a beautiful violet coloration with orcin and hydrochloric acid. The question whether this glucothionic acid originates from the above-mentioned nucleoproteid or from the mucoid substance has not been decided (LEVENE and MANDEL[1]).

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[1] Zeitschr. f. physiol. Chem., **41**.

[2] l. c., Arch. f. Math., etc.

[3] Cited from v. Gorup-Besanez, Lehrb. d. physiol. Chem., 4. Aufl., p. 732.

[4] See v. Gorup-Besanez, Lehrbuch, 4. Aufl., p. 717.

Many enzymes are found in the spleen, and certain of these are of special interest. To these belong the uric-acid-forming enzyme, the *xanthine oxidase* (BURIAN), which occurs in the spleen of oxen and horses, but not in man, dogs, and pigs (SCHITTENHELM), and which transforms the oxypurines, hypoxanthine, and xanthine into uric acid; also the hydrolytically active deamidizing enzymes *guanase* and *adenase* (LEVENE, SCHITTENHELM, JONES and PARTRIDGE, JONES and WINTERNITZ), by the first of which the guanine is transformed into xanthine, and the adenine into hypoxanthine by the latter. The guanase occurs also in the spleen of the ox and horse, but not (JONES), or only in small amounts (SCHITTENHELM), in the pig-spleen.[2] The spleen also contains two enzymes, *lienases*, as shown by HEDIN (and ROWLAND), one of which, the α-lienase, acts chiefly in alkaline solution, while the other, β-lienase, is active only in acid reaction. These enzymes not only act autolytically upon the proteins of the spleen, but they also dissolve fibrin and coagulated blood-serum. In the autolysis of the spleen LEATHES found proteoses, lysine, arginine, histidine, leucine, aminovalerianic acid, aspartic acid, and tryptophane among the cleavage products. SCHUMM[3] found, in the autolysis of a leucæmic spleen, besides leucine and tyrosine relatively large quantities of ammonia, also *r*-alanine, histidine, and lysine (but no arginine), guanine, xanthine, hypoxanthine, thymine, and *p*-lactic acid. The autolysis of the leucæmic spleen was much more extensive than the normal.

Among the constituents of the spleen the *deposit rich in iron*, which consists of ferruginous granules or conglomerate masses of them, and which is derived from a transformation of the red blood-corpuscles, is of special interest. It was closely studied by NASSE. This deposit does not occur to the same extent in the spleen of all animals. It is found especially abundant in the spleen of the horse. NASSE[4] on analyzing the grains (from the spleen of a horse) obtained 840–630 p. m. organic and 160–370 p. m. inorganic substances. These last consisted of 566–726 p. m. Fe_2O_3, 205–388 p. m. P_2O_5, and 57 p. m. earths. The organic substances consisted chiefly of proteins (660–800 p. m.), nuclein (52 p. m. maximum), a yellow coloring-matter, extractive bodies, fat, cholesterin, and lecithin.

In regard to the *mineral constituents*, it is to be observed that in comparison with sodium and phosphoric acid the amount of potassium and chlorine is small. The amount of iron in new-born and young animals is small (LAPICQUE, KRÜGER, and PERNOU), in adults more appreciable, and in old animals sometimes very considerable. NASSE found nearly 50 p. m. iron in the dried pulp of the spleen of an old horse. GUILLEMONAT and LAPICQUE[1] have determined the iron in man. They find no regular increase with growth, but in most cases 0.17–0.39 p. m. (after subtracting the blood-iron) calculated on the fresh substance. A remarkably high amount of iron is not dependent upon old age, but is a residue from chronic diseases.

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[1] Levene, Zeitschr. f. physiol. Chem., 37; Levene and Mandel, *ibid.*, 45 and 47.

[2] See Chapter XV for the literature.

[3] Hedin and Rowland, Zeitschr. f. physiol. Chem., 32, and Hedin, Journ. of Physiol., 30; Leathes, Journ. of Physiol., 28; Schumm, Hofmeister's Beiträge, 3 and 7.

[4] Maly's Jahresber., 19, p. 315.

The quantitative analyses of the human spleen by OIDTMANN[2] give the following results: In men he found 750–694 p. m. water and 250–306 p. m. solids. In that of a woman he found 774.8 p. m. water and 225.2 p. m. solids. The quantity of inorganic bodies was in men 4.9–7.4 p. m., and in women 9.5 p. m.

In regard to the pathological processes going on in the spleen we must specially recall the abundant re-formation of leucocytes in leucæmia and the appearance of amyloid substance (see page 69).

The physiological functions of the spleen are little known, with the exception of its importance in the formation of leucocytes. Some consider the spleen as an organ for the dissolution of the red blood-corpuscles, and the occurrence of the above-mentioned deposit rich in iron seems to confirm this view. The spleen has also been claimed to play a certain part in digestion. This organ is said by SCHIFF, HERZEN, and others to be of importance in the production of trypsin in the pancreas. The investigations of HERZEN seem to confirm this relation, but the recent work of PRYM[3] has made the assumption doubtful.

An increase in the quantity of uric acid eliminated in splenic leucæmia has been observed by many investigators (see Chapter XV), while the reverse has been observed under the influence of quinine in large doses, which produces an enlargement of the spleen. These facts give a rather positive proof that there is a close relationship between the spleen and the formation of uric acid. This relationship has been studied by HORBACZEWSKI. He has shown that when the spleen-pulp and blood of calves are allowed to act on each other, under certain conditions and temperature, in the presence of air, large quantities of uric acid are formed. Under other conditions he obtained from the spleen-pulp only xanthine bodies with very little or no uric acid. HORBACZEWSKI[4] has also shown that the uric acid originates from the nucleins of the spleen, which yield uric acid and xanthine bodies according to the experimental conditions. This behavior is explained by the above-mentioned investigations of BURIAN, SCHITTENHELM, JONES, and others on the enzymotic uric-acid formation and the deamidization of the purine bodies, and a relationship between the spleen and uric-acid formation is indisputable. Still we cannot say that the spleen shows a special relationship to the uric-acid formation as compared with other organs (see Chapter XV).

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[1] Lapicque, *ibid.*, **20**; Lapicque and Guillemonat, Compt. rend. de soc. biol., **48**, and Arch. de Physiol. (5), 8; Krüger and Pernou, Zeitschr. f. Biologie, **27**; Nasse, cited from Hoppe-Seyler, Physiol. Chem., 720.

[2] Cited from v. Gorup-Besanez, Lehrbuch, 4. Aufl., p. 719.

[3] Schiff, cited by Herzen, Pflüger's Arch., **30**, 295, 308, and **84**, and Maly's Jahresber., **18**; Prym, Pflüger's Arch., **104** and **107**; see also Chapter IX.

[4] Monatshefte f. Chem., **10**, and Wien. Sitzungsber. Math. Nat. Klasse, **100**, Abt. 3.

The spleen has the same property as the liver of retaining foreign bodies, metals and metalloids.

The Thyroid Gland. The nature of the different protein substances occurring in the thyroid gland has not been sufficiently studied, but at present, through the researches of OSWALD, there are known at least two bodies which are constituents of the so-called secretion of the glands. One of these, *iodothyreoglobulin*, behaves like a globulin, while the other is a nucleoproteid (see also GOURLAY [1]). The iodine present in the gland occurs chiefly in the first body, while the arsenic, which has been shown to be a normal constituent by GAUTIER and BERTRAND,[2] seems to be related to the nuclein substances.

According to OSWALD the iodothyreoglobulin occurs only in those glands which contain colloid, while the colloid-free glands, the parenchymatous goitre, and the glands of the new-born contain thyreoglobulin free from iodine. The thyreoglobulin first becomes iodized into iodothyreoglobulin on passing from the follicle-cells. Besides these mentioned bodies *leucine, xanthine, hypoxanthine, iodothyrine, lactic* and *succinic acids* occur in the thyreoidea. OIDTMANN [3] found in the thyroid gland of an old woman 822.4 p. m. water, 176.6 p. m. organic and 0.9 p. m. inorganic substances. He found 772.1 p. m. water, 223.5 p. m. organic and 4.4 p. m. inorganic substances in an infant two weeks old.

In "STRUMA CYSTICA" HOPPE-SEYLER found hardly any protein in the smaller glandular vessels, but an excess of *mucin*, while in the larger he found a great deal of *protein*, 70–80 p. m.[4] *Cholesterin* is regularly found in such cysts, sometimes in such large quantities that the entire contents form a thick mass of cholesterin plates. Crystals of *calcium oxalate* also occur frequently. The contents of the struma cysts are sometimes of a brown color due to decomposed coloring-matter, *methæmoglobin* (and hæmatin?). Bile-coloring matters have also been found in such cysts. (In regard to the *paralbumins* and *colloids* which have been found in struma cysts and colloid degeneration, see Chapter XIII.)

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[1] Gourlay, Journ. of Physiol., **16**; Oswald, Zeitschr. f. physiol. Chem., **32**, and Biochem. Centralbl., **1**, 429.

[2] Gautier, Compt. rend., **129**. See also *ibid.*, **130, 131, 134, 135**; Bertrand, *ibid.*, **134, 135**.

[3] l. c., 732.

[4] Physiol. Chem., p. 721.

Those substances which bear a close relationship to the functions of the gland seem to be of special interest.

The complete extirpation, as also the pathological destruction, of the thyroid gland causes great disturbances, ending finally in death. In dogs, after the total extripation, a disturbance of the nervous and muscular systems occurs, such as trembling and convulsions, and death generally supervenes shortly after, most often during such an attack.[1] In human beings different disturbances appear, such as nervous symptoms, diminished intelligence, dryness of the skin, falling out of the hair, and, on the whole, those symptoms which are included under the name cachexia thyreopriva, and death follows gradually. Among these symptoms must be mentioned the peculiar slimy infiltration and exuberance of the connective tissue called myxœdema. It has been proved that the destructive action of the removal of the thyroid can be counteracted by the artificial introduction of extracts of the thyroid gland into the body, and even by feeding with the substance of the gland. On the other hand, it has been observed on administering too large quantities of gland substance that threatening symptoms and disturbances occur in man as well as in animals. From a physiologico-chemical standpoint the abnormally increased destruction of body protein, occurring on continuous feeding with thyroid preparations, is of the greatest importance.

From this it follows that the glands contain specifically active substances. It is impossible for the present to state anything about the importance of the bases found by certain investigators, such as S. FRÄNKEL, DRECHSEL, and KOCHER;[2] these bodies have not been characterized sufficiently. It seems positively proven that the specifically active substance is, in greater part, if not entirely, as first shown by NOTKIN,[3] a protein substance: NOTKIN'S *thyreoproteid*, OSWALD'S *thyreoglobulin*. This does not contradict the views of BAUMANN and ROOS that the active substance is iodothyrin, as this is produced as a cleavage product from the iodothyreoglobulin.

Iodothyrin is considered by BAUMANN, who first showed that the thyroid contained iodine and who with ROOS[4] showed the importance of this substance for the physiological activity of the gland, as the only active substance. Iodothyrin was obtained by BAUMANN by boiling the finely divided gland with dilute sulphuric acid as an amorphous, brown mass nearly insoluble in water but readily soluble in alkali and precipitated again by the addition of acid. The iodothyrin, which is not a unit body, has a variable content of iodine and is not a protein substance.

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[1] The divergent statements as to the necessity of the thyroid gland can be found in H. Munk, Virchow's Arch., **150**.

[2] Fränkel, Wien. med. Blätter, 1895 and 1896; Drechsel and Kocher, Centralbl. f. Physiol., **9**, 705.

[3] Wien. med. Wochenschr., 1895, and Virchow's Arch., **144**, Suppl., 224.

[4] In regard to this subject, see Baumann and Roos, Zeitschr. f. physiol. Chem., **21** and **22**; also Baumann, Münch. med. Wochenschr., 1896; Baumann and Goldmann, *ibid.*; Roos, *ibid.* An extensive review of the literature on the action of iodothyrin

Thyreoglobulin was obtained by OSWALD from the watery extract of the gland by half saturating with ammonium sulphate. It has the properties of the globulins and with the exception of the iodine content it has about the same composition as the proteins. The amount of iodine varies: 0.46 per cent in pigs, 0.86 per cent in oxen, and 0.34 per cent in man. In young animals, whose glands contain no iodine, the thyreoglobulin is iodine-free. Thyreoglobulin on taking up iodine is converted into iodothyreoglobulin. By introducing iodine salts the iodine content of the iodothyreoglobulin can be raised in living animals and thereby also the physiological activity increased (OSWALD). The amount of iodine in the gland is markedly dependent upon the food.

According to OSWALD iodothyreoglobulin, as a physiological excitant upon the nervous system, has a regulating action upon metabolism. The exclusion of this action, after destruction or extirpation of the gland, explains, according to OSWALD, the injurious results produced by these changes upon the gland. According to BLUM the thyroid gland removes from the blood a poisonous body, the *thyreotoxalbumin*, and makes it non-injurious by taking up iodine. KISHI [1] also believes that the thyroid gland has the power of removing poisons from the blood. We cannot enter further into this and other related questions.

The Suprarenal Capsule. Besides proteins, substances of the connective tissue, and salts, there occur in the suprarenal capsule *inosite*, purine bases, especially *xanthine* (OKER-BLOM), a protagon-like substance (ORGLER), relatively considerable *lecithin* and *neurine*, and *glycerophosphoric acid*, which are probably decomposition products of the lecithin. The older statements on the occurrence of benzoic acid, hippuric acid, and bile-acids are, on the contrary, doubtful and are not substantiated by recent investigations (STADELMANN). Older investigators, like VULPIAN and ARNOLD,[2] have found in the medulla a *chromogen* which was considered to be connected with the abnormal pigmentation of the skin in Addison's disease. This chromogen, which is transformed by air, light, alkalies, iodine, and other bodies into a red pigment, seems, on the contrary, to be related to the substance of the gland producing an increase in the blood-pressure.

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and the thyroid preparations can be found in Roos, Zeitschr. f. physiol. Chem., 22, 18. In regard to their action in protein destruction and metabolism, see F. Voit, Zeitschr. f. Biologie, 35; Schöndorff, Pflüger's Arch., 67, and Andersson and Bergman, Skand. Arch. f. Physiol., 8; Magnus-Levy, Zeitschr. f. klin. Med., 52.

[1] Kishi, Virchow's Arch., 176. A summary of the thyroid literature for the last years is found in Maly's Jahresber., 24 and 25. See also the works of Blum and Oswald, cited by Oswald in Biochem. Centralbl., 1, 249.

[2] Oker-Blom, Zeitschr. f. physiol. Chem., 28; Stadelmann, *ibid.*, 18, which also contains the literature on this subject; Orgler, Salkowski's Festschrift, 1904.

Adrenalin (suprarenin, epinephrin). That the watery extract of the suprarenal capsule has a blood-pressure-raising action was shown by OLIVER and SCHÄFER, CYBULSKI and SZYMONOWICZ.[1] The substance which is here active was formerly called sphygmogenin and has also other actions besides bringing about a marked increase in blood-pressure by the strong contraction of the muscles of the periphery vessels; for instance, it can bring about glycosuria. This body has been chemically investigated by several experimenters and has received different names. v. FÜRTH calls it *suprarenin*, ABEL *epinephrin*, and TAKAMINE *adrenalin*. This last name seems to be the most generally accepted one. The chemical constitution of adrenalin has not been positively decided upon. ALDRICH gives the formula $C_9H_{13}NO_3$ for adrenalin, and this formula has been accepted as correct by a large number of investigators, such as v. FÜRTH, JOWETT, PAULY, ABDERHALDEN and BERGELL, BERTRAND, FRIEDMANN, and STOLZ, basing their opinion upon their own researches.[2] ABEL disputes the correctness of this formula and considers adrenalin as a hydrate of a substance, $C_{10}H_{13}NO_3$, called epinephrin by him, hence it is epinephrin hydrate, $C_{10}H_{13}NO_3+\frac{1}{2}H_2O$. The general opinion in regard to the constitution of adrenalin is that it contains a pyrocatechin complex, three OH groups, of which one is found in the side-chain, and one CH_3NH group. The formula $(HO)_2.C_6H_3.CH(OH).CH_2.NH.CH_3$, given by PAULY, according to the investigations of FRIEDMANN, can be accepted as correct. Based upon these facts it has been possible to prepare compounds synthetically whose physiological action was more or less similar to adrenalin, namely, by starting from pyrocatechin, especially by treating chloracetopyrocatechin with ammonia, alkylamines, and other basic bodies (STOLZ, MEYER, FRIEDMANN, DAKIN).[3]

Adrenalin is soluble in water, precipitated by ammonia, and thereby separates as crystals. It gives an emerald-green color with ferric chloride in acid solution and a carmine-red coloration in alkaline solution. It reduces Fehling's solution and an ammoniacal silver solution. Epinephrin (ABEL) is precipitated by several alkaloid reagents and gives color reactions with Mandelin's alkaloid reagent and with permanganate and sulphuric acid. On this point the conditions are not quite clear. According to ABEL the crystalline substance (his epinephrin hydrate) precipitated by ammonia, which corresponds to the adrenalin of the other investigators, does not have the alkaloid properties of epinephrin, but is converted by the action of mineral acids into epinephrin. The epinephrin is probably a transformation product of adrenalin. Further investigation is necessary before this can be explained.

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[1] Oliver and Schäfer, Proceed. of Physiol. Soc., London, 1895. Further literature on the function of the suprarenal capsule may be found in Szymonowicz, Pflüger's Arch., **64**.

[2] The literature on this subject may be found in v. Fürth, Zeitschr. f. physiol. Chem., **23**, **26**, **29**, and Wien. Sitzungsber. Math. Nat. Kl., **112**, 1903. See also Abel, Zeitschr. f. physiol. Chem., **28**; Amer. Journ. of Physiol., 1899, and The Johns Hopkins Hospital Bull., No. **76** (1897), **90** and **91** (1898), **120** and **128** (1901), **131** and **132** (1902); Ber. d. d. chem. Gesellsch., **36**; Abel and Taveau, Journ. of Biol. Chem., **1**, and Friedmann, Hofmeister's Beiträge, **6** and **8**.

[3] Stolz, Ber. d. d. chem. Gesellsch., **37**; Friedmann, Hofmeister's Beiträge, **6** and **8**; Dakin, Proc. Roy. Soc., 1905, Ser. B, Vol. **76**.

The glycosuria first observed by BLUM after the injection of the extract of the suprarenal capsule is due to an action of the adrenalin, and it is hardly possible that the diastatic enzyme found in the suprarenal capsule by CROFTAN[1] takes any part in this change.

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[1] Blum, Pflüger's Arch., 90; Croftan, *ibid.*, 90.

CHAPTER VIII.

THE LIVER.

THE liver, which is the largest gland of the body, stands in close relationship to the blood-forming glands. The importance of this organ for the physiological composition of the blood is evident from the fact that the blood coming from the digestive tract, laden with absorbed bodies, must circulate through the liver before it is driven by the heart through the different organs and tissues. It has been proved, at least for the carbohydrates, that an assimilation of the absorbed nutritive substances which are brought to the liver by the blood of the portal vein takes place in this organ, and there is no doubt that synthetical processes also occur. The occurrence of synthetical processes in the liver has been positively proved by special observations. It is possible that in the liver certain ammonia combinations are converted into urea or uric acid (in birds) (see Chapter XV), while certain products of putrefaction in the intestine, such as phenols, may be converted by synthesis into ethereal sulphuric acids by the liver (PFLÜGER and KOCHS, EMBDEN and GLAESSNER), probably also converted into conjugated glucuronic acids (EMBDEN[1]). The liver has also the property of removing and retaining heterogeneous bodies from the blood, and this is not only true of metallic salts, which are often removed by this organ, but also, as SCHIFF, HEGER, and others, but especially ROGER, have shown, the alkaloids are retained, and are probably also partially decomposed in the liver. Toxines are also withheld by the liver, and hence this organ has a protective action against poisons.[2]

Even though the liver is of assimilatory importance and purifies the blood coming from the digestive tract, it is at the same time a secretory organ which eliminates a specific secretion, the bile, in the production of which the red blood-corpuscles are destroyed, or at least one of their constituents, the hæmoglobin. It is generally admitted that the liver acts contrariwise during fœtal life, at that time forming the red blood-corpuscles.

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[1] Pflüger and Kochs, Pflüger's Arch., 20 and 23; Embden and Glaessner, Hofmeister's Beiträge, 1; Embden, *ibid.*, 2.

[2] Roger, Action du foie sur les poisons (Paris, 1887), which also contains the older literature; Bouchard, Leçons sur les autointoxications dans les maladies (Paris, 1887); and E. Kotliar in Arch. des sciences biologiques de St. Pétersbourg, 2. See also de Vamossy, Centralbl. f. Physiol., 18, and Rothberger, Wien. klin. Wochenschr., 1905, Rothberger and Winterberg, Biochem. Centralbl., 4.

There is no doubt that the chemical operations going on in this organ are manifold and must be of the greatest importance for the organism. Our knowledge on this subject has been essentially advanced by the recent investigations on the enzymes of the liver, as well as on the autolytic processes in this organ,[1] but nevertheless it must be admitted that our knowledge of the character and extent of these changes is still small. Among the products of these chemical processes there are two which are especially important and must be treated in this chapter, namely, the glycogen and the bile. Before the study of these products is taken up a short discussion of the constituents and the chemical composition of the liver is necessary.

The reaction of the liver-cells is alkaline towards litmus during life, but becomes acid after death, due to a formation of lactic acid, chiefly fermentation lactic acid and other organic acids (MORISHIMA, MAGNUS-LEVY[2]). A coagulation of the protoplasmic proteins in the cells probably takes place. A positive difference between the proteins of the dead and the living, non-coagulated protoplasm has not been observed.

The *proteins* of the liver were first carefully investigated by PLÓSZ. He found in the watery extract of the liver an *albuminous substance* which coagulates at 45° C., also a *globulin* which coagulates at 75° C., a *nucleoalbumin* which coagulates at 70° C., and lastly a protein body which is nearly related to the *coagulated albumins* and which is insoluble in dilute acids or alkalies at the ordinary temperature, but dissolves on the application of heat, being converted into an albuminate. HALLIBURTON[3] has found two globulins in the liver-cells, one of which coagulates at 68–70° C., and the other at 45–50° C. He also found, besides traces of albumin, a nucleoproteid which possessed 1.45 per cent phosphorus and a coagulation-point of 60° C. POHL has obtained an "organ plasma" by extracting the finely divided liver which had previously been entirely freed from blood by washing with 8 p. m. NaCl solution, in which he was able to detect a globulin having a low coagulation temperature. The very variable phosphorus content (0.28–1.3 per cent) of this globulin as well as the insolubility of the precipitates produced by little acid in an excess of acid and in neutral salts seem to indicate that we have here a mixture which consists chiefly of nucleoproteids and not of globulins. The nearly complete digestibility with pepsin-hydrochloric acid does not contradict this assumption, because, as is known, nucleoproteids may on digestion yield no residue (see Chapter V). It is also impossible to state anything positive about the nature of the liver-globulin found by DASTRE,[1] having a coagulation temperature of 56°. The proteins extractable from the liver without modification must be thoroughly investigated.

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[1] See especially the works of Jacoby, Zeitschr. f. physiol. Chem., **30**; Conradi, Hofmeister's Beiträge, **1**; Magnus-Levy, *ibid.*, **2**.

[2] Morishima, Arch. f. exp. Path. u. Pharm., **43**; Magnus-Levy, l. c.

[3] Plósz, Pflüger's Arch., **7**; Halliburton, Journ. of Physiol., **13**, Suppl. 1892.

Besides the above-mentioned proteins which are very soluble, the liver-cells contain large quantities of difficultly soluble protein bodies (see PLOSZ). The liver also contains, as first shown by ST. ZALESKI and then substantiated by several other investigators, ferruginous proteins of different kinds.[2] The chief portion of the protein substances in the liver seems to consist in fact of ferruginous nucleoproteids. On boiling the liver with water, such a nucleoproteid or perhaps several are split, and a solution is obtained containing a nucleic-acid-rich nucleoproteid or a mixture of these which are precipitable by acids. This protein or protein mixture, which has been called *ferratin* by SCHMIEDEBERG,[3] has been carefully studied by WOHLGEMUTH.[4] The quantity of phosphorus was 3.06 per cent. As cleavage products on hydrolysis he found *l*-xylose, the four nuclein bases, and also arginine, lysine (and histidine?), tyrosine, leucine, glycocoll, alanine, α-proline, glutamic acid, aspartic acid, phenylalanine, oxyaminosuberic acid, and oxydiaminosebacic acid (see Chapter II).

The yellow or brown pigment of the liver has been little studied. DASTRE and FLORESCO[5] differentiate in vertebrates and certain invertebrates between a ferruginous pigment soluble in water, *ferrine*, and a pigment soluble in chloroform and insoluble in water, *chlorochrome*. They have not isolated these pigments in a pure condition. In certain invertebrates chlorophyll originating from the food also occurs in the liver.

The *fat* of the liver occurs partly as very small globules and partly (especially in nursing children and sucking animals, as also after food rich in fat) as rather large fat-drops. The occurrence of a fatty infiltration, i.e., a transportation of fat to the liver, may not only be produced by an excess of fat in the food (NOËL-PATON), but also by a migration from other parts of the body under abnormal conditions, such as posioning with phosphorus, phlorhizin, and certain other bodies (LEO, LEBEDEFF, ROSENFELD, and others[1]). The fatty infiltration occurring in poisoning and which is accompanied with degenerative changes in the cells may cause a diminution in the amount of protein and a rise in the water content. If the amount of fat in the liver is increased by an infiltration, the water decreases correspondingly, while the quantity of the other solids remains little changed. Changes of such a kind may occur, so that, because of the opposition (ROSENFELD) existing between glycogen and fat, a liver rich in fat is habitually poor in glycogen. The reverse occurs after feeding with carbohydrate-rich food, namely, the liver is rich in glycogen and poor in fat.

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[1] Pohl, Hofmeister's Beiträge, **7**; Dastre, Compt. rend. soc. biolog., **58**.

[2] St. Zaleski, Zeitschr. f. physiol. Chem., **10**, 486; Woltering, *ibid.*, **21**; Spitzer, Pflüger's Arch., **67**.

[3] Arch. f. exp. Path. u. Pharm., **33**; see also Vay, Zeitschr. f. physiol. Chem., **20**.

[4] Wohlgemuth, Zeitschr. f. physiol. Chem., **37**, **42**, and **44**, and Ber. d. d. chem. Gesellsch., **37**. See on liver nucleoproteids also Salkowski, Berl. klin. Wochenschr., 1895; Hammarsten, Zeitschr. f. physiol. Chem., **19**; Blumenthal, Zeitschr. f. klin. Med., **34**.

[5] Arch. de Physiol. (5), **10**.

The composition of the liver-fat not only seems to vary in different animals, but is variable with changing conditions. Thus NOËL-PATON found that the liver-fat in man and several animals was poorer in oleic acid and had a correspondingly higher melting-point than the fat from the subcutaneous connective tissue, while ROSENFELD[2] has observed the opposite condition on feeding dogs with mutton-fat.

Lecithin is a normal constituent of the liver, and amounts to about 23.5 p. m. according to NOËL-PATON.[3] In starvation the lecithin, according to NOËL-PATON, forms the greatest part of the ethereal extract, while with food rich in fat, on the contrary, it forms the smallest part. *Cholesterin* occurs only in small quantities. The ethereal extract also contains a protagon-like body, *jecorin.*

Jecorin was first found by DRECHSEL in the liver of horses, and also in the liver of a dolphin, and later by BALDI in the liver and spleen of other animals, in the muscles and blood of the horse, and in the human brain. It contains sulphur and phosphorus, but its constitution is not positively known. Jecorin dissolves in ether, but is precipitated from this solution by alcohol. It reduces copper oxide, and it solidifies after boiling with alkalies to a gelatinous mass. MANASSE has detected dextrose as osazone in the carbohydrate complex of jecorin. It may lead to errors in the investigations of organs or tissues, for it can easily be mistaken for lecithin on account of its solubilities and because it contains phosphorus.

The statement by BING that jecorin is a combination of lecithin and dextrose does not follow from the analyses of jecorin thus far known. Jecorin contains sulphur, even as much as 2.75 per cent, and also the relation of P:N in lecithin is 1:1, while in jecorin it is quite different, 1:2 to 1:6.

The variable composition and divergent properties of the jecorin isolated and analyzed by various investigators[4] make it very possible that jecorin is a mixture of several substances, among which perhaps occurs a sulphurized and phosphorized substance (SIEGFRIED and MARK).

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[1] Noël-Paton, Journ. of Physiol., 19; Leo, Zeitschr. f. physiol. Chem., 9; Lebedeff, Pflüger's Arch., 31; Athanasiu, Pfluger's Arch., 74; Taylor, Journ. of Exp. Med., 4; Kraus u. Sommer, Hofmeister's Beiträge, 2; Rosenfeld, Zeitschr. f. klin. Med., 36. See also Rosenfeld, Ergebnisse der Physiologie, 1, Abt. 1, and Berl. klin. Wochenschr., 1904; Schwalbe, Centralbl. f. Physiol., 18, p. 319.

[2] Cited by Lummert, Pflüger's Arch., 71. In regard to the liver-fat of children, see Thiemich, Zeitschr. f. physiol. Chem., 26.

[3] l. c. See also Hefter, Arch. f. exp. Path. u. Pharm., 28.

[4] Drechsel, Ber. d. sächs. Gesellsch. d. Wissensch., 1886, p. 44, and Zeitschr. f. Biologie, 33; Baldi, Arch. f. (Anat. u.) Physiol., 1887, Suppl. 100; Manasse, Zeitschr. f. physiol. Chem., 20; Bing, Centralbl. f. Physiol., 12, and Skand. Arch. f. Physiol., 9; Meinertz, Zeitschr. f. physiol. Chem., 46; Siegfried and Mark, *ibid*

Among the *extractive substances* besides *glycogen*, which will be treated later, rather large quantities of the *xanthine bodies* occur. KOSSEL[1] found in 1000 parts of the dried substance 1.97 p. m. *guanine*, 1.34 p. m. *hypoxanthine*, and 1.21 p. m. *xanthine*. *Adenine* is also contained in the liver. In addition there have been found *urea* and *uric acid* (especially in birds), and indeed in larger quantities than in the blood, *paralactic acid*, *leucine*, and *cystine*. In pathological cases *inosite* and *tyrosine* have been detected. The occurrence of *bile-coloring matters* in the liver-cell under normal conditions is doubtful; but in retention of the bile the cells may absorb the coloring-matter and become colored thereby.

A large number of enzymes are found in the liver, among which (besides the *catalases*, the *oxidases*, the *glycolytic enzyme*, which will be spoken of later, the enzymes taking part in the *formation of uric acid* and *destruction of uric acid* (Chapter XV), the *arginase* which forms *urea*, and the *diastase* acting upon glycogen) we must mention the so-called *lipase* and the *proteolytic enzyme*. The liver has the power of splitting various esters, an action which has been recently studied by DAKIN,[2] and this action is due to an enzyme which is considered as a lipase. The nature of this lipase, whose cleavage action upon the amyl ester of salicylic acid was first observed by CHANOZ and DOYEN, has been closely studied by MAGNUS,[3] and it has been shown that this action is the result of the combined action of two substances. The lipase solution becomes inactive by dialysis, a thermostable substance soluble in absolute alcohol passing into the diffusate, and this body acts as a co-enzyme, making the solution which had been made inactive by dialysis active again.

The proteolytic enzymes of the liver are of special interest, especially in regard to the study of the autolysis of this organ. The processes in the liver in phosphorus poisoning and in acute yellow atrophy of the liver are considered as an intravitally increased autolysis. In these cases a softening of the organ takes place, and proteoses, mon- and diamino-acids, and other bodies are produced, which may also in part be found in the urine, and although they may not all be derived from the liver (NEUBERG and RICHTER), are at least in part derived from this organ.

WAKEMAN[4] has found in phosphorus poisoning that not only is the quantity of nitrogen markedly diminished in the liver (of dogs), but also that the quantity of nitrogen of the hexone bases is diminished, and that the part of the protein molecule richer in nitrogen is first removed and eliminated under these conditions. The increased consumption of glycogen under the above-mentioned pathological conditions may also be considered as an increased autolysis.

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[1] Zeitschr. f. physiol. Chem., 8.

[2] Journ. of Physiol., **30** and **32**.

[3] Chanoz and Doyen, Journ. de physiol. et de path. général, **2**; Magnus, Zeitschr. f. physiol. Chem., **42**.

[4] Neuberg and Richter, Deutsch. Med. Wochenschr., 1904; Wakeman, Zeitschr. f. physiol. Chem., **44**.

Besides the above-mentioned organic constituents in the liver we must mention the *glucothionic acid* found by MANDEL and LEVENE, whose relationship to the carbohydrate metabolism in this organ, as well as to the nitrogenous carbohydrate found by SEEGEN and NEIMANN[1] in the liver, requires further investigation.

The *mineral bodies* of the liver consist of phosphoric acid, potassium, sodium, alkaline earths, and chlorine. The potassium is in excess of the sodium. Iron is a regular constituent of the liver, but it occurs in very variable amounts. BUNGE has found 0.01–0.355 p. m. iron in the blood-free liver of young cats and dogs. This was calculated on the liver substance freshly washed with a 1 per cent NaCl solution. Calculated on 10 kilos bodily weight, the iron in the liver amounted to 3.4–80.1 mg. Recent determinations of the quantity of iron in the liver of the rabbit, dog, hedgehog, pig, and man have been made by GUILLEMONAT and LAPICQUE. The variation was great in human beings. In men the quantity of iron in the blood-free liver (blood-pigment subtracted in the calculation) was regularly more, and in women less, than 0.20 p. m. (calculated on the fresh moist organ). Above 0.5 p. m. is considered as pathological. According to BIELFELD,[2] who also finds a greater iron content in men, this difference appears only after the first 20–25 years. At this age (20–25 years) the iron content is smallest.

The quantity of iron in the liver can be increased by drugs containing iron, as also by inorganic iron salts, and the largest deposition of iron was observed by NOVI[3] after the hypodermic injection of iron. The quantity of iron may also be increased by an abundant destruction of red blood-corpuscles, which will result from the injection of dissolved hæmoglobin, in which process the iron combinations derived from the blood-pigments in other organs, such as the spleen and marrow, also seem to take part.[4] A destruction of blood-pigments, with a splitting off of compounds rich in iron, seems to take place in the liver in the formation of the bile-pigments. Even in invertebrates, which have no hæmoglobin, the so-called liver is rich in iron, from which DASTRE and FLORESCO[1] conclude that the quantity of iron in the liver of invertebrates is entirely independent of the decomposition of the blood-pigment, and in vertebrates it is in part so. According to these authors the liver has, on account of the quantity of iron, a specially important oxidizing function, which they call the "*fonction martiale*" of the liver.

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[1] Mandel and Levene, Zeitschr. f. physiol. Chem., 45; Seegen, Centralbl. f. Physiol., 12 and 13, with Neimann, Wiener Sitzungsber. Math. Klasse, 112.

[2] Bunge, Zeitschr. f. physiol. Chem., 17, 78; Guillemonat and Lapicque, Compt. rend. de soc. biol., 48, and Arch. de Physiol. (5), 8; Bielfeld, Hofmeister's Beiträge, 2; see also Schmey, Zeitschr. f. physiol. Chem., 39.

[3] See Centralbl. d. Physiol., 16, 393.

[4] See Lapicque, Compt. rend., 124, and Schurig, Arch. f. exp. Path. u. Pharm., 41.

The richness in iron of the liver of new-born animals is of special interest—a condition which was shown by the analyses of ST. ZALESKI, but was especially studied by KRÜGER and MEYER. In oxen and cows they found 0.246–0.276 p. m. iron (calculated on the dry substance), and in the cow-fœtus about ten times as much. The liver-cells of a calf a week old contain about seven times as much iron as the adult animal; the quantity sinks in the first four weeks of life, when it reaches about the same amount as in the adult. LAPICQUE[2] has also found that in rabbits the quantity of iron in the liver steadily diminishes from the eighth day to three months after birth, namely, from 10 to 0.4 p. m., calculated on the dry substance. "The fœtal liver-cells bring an abundance of iron into the world to be used up, within a certain time, for a purpose not well known." A part of the iron exists as phosphate, but the greater part is in combination in the ferruginous protein bodies (ST. ZALESKI).

The quantity of calcium oxide in the fresh, moist liver of the horse, ox, and pig, according to TOYONAGA, amounts to 0.148–0.193 p. m., or about the same as in the human liver. The amount of magnesium oxide was remarkably high, namely, 0.168, 0.198, and 0.158 p. m., in the livers of the horse, ox, and pig respectively. KRÜGER[3] has found the quantity of calcium in the livers of adult cattle and of calves to be respectively 0.71 p. m. and 1.23 p. m. of the dried substance. In the fœtus of the cow it is lower than in calves. During pregnancy the iron and calcium in the fœtus are antagonistic; that is, an increase in the quantity of calcium in the liver causes a diminution in the iron, and an increase in the iron causes a decrease in the calcium. Copper seems to be a physiological constituent, and occurs to a considerable extent in cephalopods (HENZE).[4] Foreign metals, such as lead, zinc, and others (also iron), are easily taken up and combined by the liver (SLOWTZOFF, v. ZEYNEK, and others[5]).

v. BIBRA[6] found in the liver of a young man who had suddenly died 762 p. m. water and 238 p. m. solids, consisting of 25 p. m. fat, 152 p. m.

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[1] Arch. de Physiol. (5), **10**.

[2] St. Zaleski, l. c.; Krüger and collaborators, Zeitschr. f. Biologie, **27**; Lapicque, Maly's Jahresber., **20**.

[3] Zeitschr. f. Biologie, **31**; Toyonaga, Bull. of the College of Agriculture, Tokio, **6**.

[4] Zeitschr. f. physiol. Chem., **33**.

[5] Slowtzoff, Hofmeister's Beiträge, **1**; v. Zeynek, see Centralbl. f. Physiol., **15**.

[6] See v. Gorup-Besanez, Lehrbuch d. physiol. Chem., 4. Aufl., p. 711.

protein, gelatine-forming and insoluble substances, and 61 p. m. extractive substances.

The quantitative composition of the liver may show great variation, depending upon the kind and amount of the food supplied. The amount of carbohydrate (glycogen) and fat may vary considerably, which is due to the fact that the liver is a storage-organ for these bodies, especially for the glycogen.

Based upon special experiments, SEITZ[1] claims that the liver is a storehouse also for protein. In experiments on hens and ducks which had previously been starved, he found that the liver took up abundant protein on feeding meat and that its weight as compared with the weight after starvation was doubled or quadrupled. As it is characteristic of storage or reserve bodies that their amount in the storage-organs on feeding with such bodies strongly increases in percentage, it is remarkable in SEITZ's feeding experiments that the percentage of protein in the liver did not increase but rather diminished slightly. In this case we did not have a higher percentage of protein, but an increase in the weight of the total cell mass of the organ, probably brought about by increased work of the liver due to the protein feeding. It is also difficult to decide as to how far in these experiments we were dealing with an increase in the number or the size of the liver-cells or with a deposition of reserve protein in the same sense as of glycogen or excessive fat.

There is an unanimous belief that the liver is an especially important storage-organ for glycogen.

Glycogen and its Formation.

Glycogen was first discovered by BERNARD. It is a carbohydrate closely related to the starches or dextrins, with the general formula $(C_6H_{10}O_5)_x$. Its molecular weight is unknown, but seems to be very large (GATIN-GRUZEWSKA and v. KNAFFL-LENZ[2]). The largest quantities are found in the liver, and smaller quantities in the muscles (BERNARD, NASSE). It is found in very small quantities in nearly all tissues of the animal body. Its occurrence in lymphoid cells, blood, and pus has been mentioned in a previous chapter, and it seems to be a regular constituent of all cells capable of development. Glycogen was first shown to exist in embryonic tissues by BERNARD and KÜHNE, and it seems on the whole to be a constituent of tissues in which a rapid cell formation and cell development is taking place. It is also present in rapidly forming pathological swellings (HOPPE-SEYLER). Certain animals, as certain mussels (BIZIO), tænia and ascarides

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[1] Pflüger's Arch., 111.

[2] Gatin-Gruzewska, Pflüger's Arch., 103; v. Knaffl-Lenz, Zeitschr. f. physiol. Chem., 46.

(Weinland[1]), are very rich in glycogen. Glycogen also occurs in the vegetable kingdom, especially in many fungi.

The quantity of glycogen in the liver, as also in the muscles, depends essentially upon the food. In starvation it disappears nearly completely after a short time, but more rapidly in small than in large animals, and it disappears earlier from the liver than from the muscles. After partaking of food, especially such as is rich in carbohydrates, the liver becomes rich again in glycogen, the greatest increment occurring 14 to 16 hours after eating (Külz). The quantity of liver-glycogen may amount to 120–160 p. m. after partaking of large quantities of carbohydrates, and in dogs which had been especially fed on glycogen Schöndorff and Gatin-Gruzewska found still higher results, even more than 180 p. m. Ordinarily it is considerably less, namely, 12–30 to 40 p. m. According to Cremer the quantity of glycogen in plants (yeast-cells) is, as in animals, dependent upon the food. According to him the yeast-cells contain glycogen, which disappears from the cells in the auto-fermentation of the yeast, but reappears on the introduction of the cells into a sugar solution.

The quantity of glycogen of the liver (and also of the muscles) is also dependent upon rest and activity, because during rest, as in hibernation, it increases, and during work it diminishes. Külz has shown that by hard work the quantity of glycogen in the liver (of dogs) is reduced to a minimum in a few hours. The muscle-glycogen does not diminish to the same extent as the liver-glycogen. Külz, Zuntz and Vogelius, Frentzel, and others have been able to render rabbits and frogs glycogen-free by suitable strychnine poisoning. The same result is produced by starvation followed by hard work.

Glycogen forms an amorphous, white, tasteless, and inodorous powder. When perfectly pure and by proper alcohol precipitation it can be obtained as rods or prisms which look like crystals (Gatin-Gruzewska). It gives an opalescent solution with water which, when allowed to evaporate on the water-bath, forms a pellicle over the surface that disappears again on cooling. It is undecided whether we have here a true solution or not. Like other colloids, glycogen in water under the influence of the electric current migrates to the anode, on which it collects (Gatin-Gruzewska). Its aqueous solution is dextrorotatory, and Huppert found it to be $(\alpha)_D = +196.63°$. Gatin-Gruzewska has recently obtained the same result by using a perfectly pure solution of glycogen. A solution of glycogen, especially on the addition of NaCl, is colored wine-red by iodine. It may hold cupric hydrate in solution in alkaline liquids, but does not reduce it. A solution of glycogen in water is not precipitated by potassium-mercuric iodide and hydrochloric acid, but is precipitated by alcohol (on the addition of NaCl when necessary) or ammoniacal basic lead acetate. An aqueous solution of glycogen made alkaline with caustic potash (15 per cent KOH) is completely precipitated by an equal volume of 96 per cent alcohol. Tannic acid also precipitates glycogen. It gives a white granular precipitate of benzoyl glycogen with benzoyl chloride and caustic soda. Glycogen is completely precipitated by saturating its solution at ordinary temperatures with magnesium or ammonium sulphate. It is not precipitated by sodium chloride or by half saturation with ammonium sulphate (NASSE, NEUMEISTER, HALLIBURTON, YOUNG[1]). On boiling with dilute caustic potash (1-2 per cent) the glycogen may be more or less changed, especially if it has been previously exposed to the action of acid or of BRÜCKE'S reagent (see below) (PFLÜGER). On boiling with stronger caustic potash (even of 36 per cent) it is not injured (PFLÜGER). By diastatic enzymes glycogen is converted into maltose or dextrose, depending upon the nature of the enzyme. It is transformed into dextrose by dilute mineral acids. According to TEBB[2] various dextrins appear as intermediary steps in the saccharification of glycogen, depending on whether the hydrolysis is caused by mineral acids or enzymes. The question whether the glycogen from various animals and different organs is the same in this regard has not been sufficiently investigated. Nor has it been decided whether all the glycogen in the liver occurs as such or whether it is in part combined with protein (PFLÜGER-NERKING). The recent investigations of LOESCHCKE[3] have shown that we have no positive reasons for this assumption.

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[1] Zeitschr. f. Biologie, **41**. The extensive literature on glycogen may be found in E. Pflüger, Glykogen, 2. Aufl., Bonn, 1905; and in Cremer, "Physiol. des Glykogens," in Ergebnisse der Physiologie, **1**, Abt. 1. In the following pages we shall refer to these works.

The preparation of pure glycogen (most easily from the liver) is generally performed by the method suggested by BRÜCKE, of which the main points are the following: Immediately after the death of the animal the liver is thrown into boiling water, then finely divided and boiled several times with fresh water. The filtered extract is now sufficiently concentrated, allowed to cool, and the proteins removed by alternately adding potassium-mercuric iodide and hydrochloric acid. The glycogen is precipitated from the filtered liquid by the addition of alcohol until the liquid contains 60 vols. per cent. By repeating this and precipitating the glycogen several times from its alkaline and acetic-acid solution it is purified on the filter by washing first with 60 per cent and then with 95 per cent alcohol, then treating with ether and drying over sulphuric acid. It is always contaminated with mineral substances. To be able to extract the glycogen from the liver or, especially, from muscles and other tissues completely, which is essential in a quantitative estimation, these parts must first be warmed for two hours with strong caustic potash (30 per cent) on the water-bath. As the glycogen changes in this purification, according to BRÜCKE, it is better, for quantitative determinations of glycogen, to precipitate it directly from the alkaline solution by alcohol (PFLÜGER[1]).

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[1] Young, Journ. of Physiol., 22, citing the other investigators.

[2] Journ. of Physiol., 22.

[3] Pflüger's Arch., 102.

The quantitative estimation is best performed according to PFLÜGER'S method, which is based upon the following: 100 grams of the finely divided organ and 100 c.c. of 60 per cent caustic-potash solution are heated on the water-bath for two hours. After diluting with water to 400 c.c. it is filtered through glass wool and the glycogen precipitated from 100 c.c. of the filtrate by 100 c.c. of 96 per cent alcohol. The glycogen is washed on the filter first with dilute alkali and alcohol and then with alcohol alone. It is then dissolved in water, exactly neutralized, treated with 25 c.c. hydrochloric acid (1.19 sp. gr.) and water added to 500 c.c., when the amount of HCl will be 2.2 per cent. On heating for three hours the glycogen will have been converted into dextrose, whose quantity can be determined according to ALLIHN-PFLÜGER'S method by reduction of an alkaline copper solution and weighing the cuprous oxide. As a control the weighed cuprous oxide is dissolved in nitric acid and the copper titrated according to VOLHARD'S method. In regard to the detailed steps, which must be exactly observed, compare PFLÜGER'S original work. Other methods of estimating glycogen, such as those of BRÜCKE-KÜLZ, PAVY, and AUSTIN, are described in PFLÜGER'S Archiv, **96**. See also the new method as suggested by SALKOWSKI and the short quantitative analysis of glycogen by PFLÜGER.[2]

Numerous investigators have endeavored to determine the origin of glycogen in the animal body. It is positively established by the unanimous observations of many investigators[3] that the varieties of *sugars* and their anhydrides, *dextrins* and *starches*, have the property of increasing the quantity of glycogen in the body. The action of inulin seems to be somewhat uncertain.[4] The statements are questioned in regard to the action of the pentoses. CREMER found in rabbits and hens that various pentoses, such as rhamnose, xylose, and arabinose, have a positive influence on the glycogen formation, and SALKOWSKI obtained the same result on feeding *l*-arabinose. FRENTZEL found, on the contrary, no glycogen formation on feeding xylose to a rabbit which had previously been made glycogen-free by strychnine poisoning, and NEUBERG and WOHLGEMUTH[5] obtained similar negative results on feeding rabbits with *d*- and *r*-arabinose.

The hexoses, and the carbohydrates derived therefrom, do not all possess the ability of forming or accumulating glycogen to the same extent. Thus

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[1] See also the method suggested by Gautier, Compt. rend., **129**.

[2] Salkowski, Zeitschr. f. physiol. Chem., **36**; Pflüger, Pflüger's Arch., **103**.

[3] In reference to the literature on this subject, see E. Külz, Pflüger's Arch., **24**, and Ludwig-Festschrift, 1891; also the cited works of Pflüger and Cremer, foot-note 1, . 288.

[4] See Miura, Zeitschr. f. Biologie, **32**, and Nakaseko, Amer. Journ. of Physiol., **4**.

[5] Salkowski, Zeitschr. f. physiol. Chem., **32**; Neuberg and Wohlgemuth, *ibid.*, **35**. See also Pflüger, l. c., and Cremer, l. c.

C. VOIT[1] and his pupils have shown that dextrose has a more powerful action than cane-sugar, while milk-sugar is less active (in rabbits and hens) than dextrose, levulose, cane-sugar, or maltose. The following substances when introduced into the body also increase the quantity of glycogen in the liver: *glycerine*, *gelatine*, *arbutin*, and likewise, according to the investigations of KÜLZ, *erythrite*, *quercite*, *dulcite*, *mannite*, *inosite*, *ethylene* and *propylene glycol*, *glucuronic anhydride*, *saccharic acid*, *mucic acid*, *sodium tartrate*, *saccharine*, *isosaccharine*, and *urea*. *Ammonium carbonate*, *glycocoll*, and *asparagine* may similarly, according to RÖHMANN, cause an increase in the amount of glycogen in the liver. According to NEBELTHAU other ammonium salts and some of the amides, as well as certain *narcotics*, *hypnotics*, and *antipyretics*, produce an increase in the glycogen of the liver. This action of the antipyretics (especially antipyrine) had been shown by LÉPINE and PORTERET.[2]

PFLÜGER has conclusively shown that we have no positive proofs as to the action of these various bodies as glycogen-formers. That glycerine may in a positive sense influence the amount of glycogen in the liver is not to be doubted from the experiments of WEISS and LUCHSINGER on glycogen formation, which will be mentioned in connection with the experiments on the relationship of glycerine to the sugar formation.

The fats, according to BOUCHARD and DESGREZ, increase the glycogen content of the muscles but not of the liver, and, according to COUVREUR,[3] the glycogen is increased at the expense of the fat in the silkworm larva as it changes into a chrysalis. In general it is believed that fat does not increase the amount of glycogen in the liver or in the animal body, although a carbohydrate formation from glycerine, but not a glycogen formation, is probable. PFLÜGER explains this by the fact that the extent of fat metabolism is not dependent upon the quantity of fat supplied, but upon the amount of fat required conditioned by work. If more fat is supplied, then it is not destroyed, but is stored up. Even when sugar is continuously formed from the fat in metabolism this is immediately burned and does not yield any material for the formation of the reserve substance glycogen.

The views in regard to the influence of the proteins are somewhat contradictory. From several investigations the conclusion has been drawn that the proteins cause an increase in the glycogen of the liver. Amongst these investigations must be included certain feeding experiments with boiled beef (NAUNYN) or blood-fibrin (v. MERING), and especially the very careful experiments made by E. KÜLZ on hens, with pure proteins, such as casein, seralbumin, and ovalbumin. The value of these experiments is disputed by PFLÜGER, and as a direct proof against the formation of glycogen from protein he refers to SCHÖNDORFF's investigations when feeding carbohydrate-free protein (casein) to frogs without finding the least increase in the total glycogen. Later BLUMENTHAL and WOHLGEMUTH arrived at similar results. They found no glycogen accumulation in frogs after feeding with casein or gelatine, but did find it after feeding with ovalbumin, which contains a carbohydrate group. On the contrary, BENDIX was able to show an increase in the glycogen in dogs by feeding casein and gelatine, as well as ovalbumin, and in fact a greater increase by casein than by ovalbumin. STOOKEY[1] arrived at similar results in hens as he found a glycogen formation after feeding casein, while he obtained no positive results after feeding glucoproteids. It seems as if the conditions in cold-blooded animals were different from those in warm-blooded ones. According to PFLÜGER, the experiments of BENDIX are not conclusive, and he doubts the formation of glycogen from protein. He claims it is only formed from carbohydrates or from the carbohydrate complex of the glucoproteids.

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[1] Zeitschr. f. Biologie, 28.

[2] Röhmann, Pflüger's Arch., 39; Nebelthau, Zeitschr. f. Biologie, 28; Lépine and Porteret, Compt. rend., **107**.

[3] Bouchard et Desgrez, Compt. rend., **130**; Couvreur, Compt. rend. de soc. biol., **47**.

Many investigators are still of the opinion that an increase in the glycogen of the liver as well as of other organs can be brought about by feeding animals with carbohydrate-free proteins.

If the question is raised as to the action of the various bodies on the accumulation of glycogen in the liver, it must be recalled that a formation of glycogen takes place in this organ, as well as a consumption of the same. An accumulation of glycogen may be caused by an increased formation of glycogen, but also by a diminished consumption, or by both.

It is not known how the various bodies above mentioned act in this regard. Certain of them probably have a retarding action on the transformation of glycogen in the liver, while others perhaps are more combustible and in this way protect the glycogen. Some probably excite the liver-cells to a more active glycogen formation, while others yield material from which the glycogen is formed and are *glycogen-formers* in the strictest sense of the word. The knowledge of these last-mentioned bodies is of the greatest importance in the question as to the origin of glycogen in the animal body, and the chief interest attaches itself to the question: To what extent are the two chief groups of food, the proteins and carbohydrates, glycogen-formers?

The great importance of the carbohydrates in the formation of glycogen has given rise to the opinion that the glycogen in the liver is produced from sugar by a synthesis in which water separates with the formation of an anhydride (Luchsinger and others). This theory (*anhydride theory*) has found opponents because it neither explains the formation of glycogen from such bodies as proteins, carbohydrates, gelatine, and others, nor the circumstance that the glycogen is always the same independent of the properties of the carbohydrate introduced, whether it is dextrogyrate or levogyrate. It used to be the opinion of many investigators that all glycogen is formed from protein, and that this splits into two parts, one containing nitrogen and the other being free from nitrogen: the latter is the glycogen. According to these views, the carbohydrates act only in that they spare the protein and the glycogen produced therefrom (*sparing theory* of Weiss, Wolffberg, and others[1]).

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[1] Schöndorff, Pflüger's Arch., 82 and 88; Blumenthal and Wohlgemuth, Berl. klin. Wochenschr., 1901; Bendix, Zeitschr. f. physiol. Chem., **32** and **34**; Stookey, Amer. Journ. of Physiol., **9**.

In opposition to this theory C. and E. Voit and their pupils have shown that the carbohydrates are "true" glycogen-formers. After partaking of large quantities of carbohydrates the amount of glycogen stored up in the body is sometimes so great that it cannot be covered by the proteids decomposed during the same time, and in these cases a glycogen formation from the carbohydrates must be admitted. According to Cremer only the fermentable sugars of the six carbon series or their di- and polysaccharides are *true glycogen-formers*. For the present, only dextrose, levulose, galactose (Weinland[2]), and perhaps also *d*-mannose (Cremer) are designated as true glycogen-formers. Other monosaccharides may indeed, according to Cremer, influence the formation of glycogen, but they are not converted into glycogen and hence are called only *pseudoglycogen-formers*.

The poly- and disaccharides may, after a cleavage into the corresponding fermentable monosaccharides, serve as glycogen-formers. This is true for at least cane-sugar and milk-sugar, which must first be inverted in the intestine. These two varieties of sugar, therefore, cannot, like dextrose and levulose, serve as glycogen-formers after subcutaneous injection, but reappear almost entirely in the urine (Dastre, Fr. Voit). Maltose, which is inverted by an enzyme present in the blood, passes only to a slight extent into the urine (Dastre and Bourquelot and others), and it can, like the monosaccharides, even after subcutaneous injection, be used in the formation of glycogen (Fr. Voit[3]).

After Pavy[4] showed the glucoproteid nature of ovalbumin and, as discussed later, that glucosamine could be split off from ovalbumin as well as from certain other protein substances (see Chapter II), the question arose whether the amino-sugar could serve in the formation of glycogen. The investigations carried out in this direction by FABIAN, FRÄNKEL and OFFER, CATHCART and BIAL,[1] have shown that the glucosamine introduced into the organism is in part eliminated unchanged in the urine and has no glycogen-forming action. No definite conclusions can be drawn from this on the behavior of the carbohydrate groups which exist not as free groups but combined with the protein molecules.

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[1] In regard to these two theories, see especially Wolffberg, Zeitschr. f. Biologie, 16.

[2] E. Voit, Zeitschr. f. Biologie, 25, 543, and C. Voit, *ibid.*, 28. See also Kausch and Socin, Arch. f. exp. Path. u. Pharm., 31; Weinland, Zeitschr. f. Biologie, 40 and 38; Cremer, *ibid.*, 42, and Ergebnisse der Physiol., 1.

[3] Dastre, Arch. de Physiol. (5), 3, 1891; Dastre and Bourquelot, Compt. rend., 98; Fritz Voit, Verhandl. d. Gesellsch. f. Morph. u. Physiol. in München, 1896, and Deutsch. Arch. f klin. Med., 58.

[4] The Physiology of the Carbohydrates, London, 1894.

Whether or not, or to what extent, the glucoproteids take part in the sugar or glycogen formation in the animal body is difficult to answer for the present, as but little is known of the quantity of these substances in the body and our knowledge of the amount of carbohydrate which can be split off from the various protein substances is also very meagre.

From the weight of the various organs and the relationship of the weight of the organs to the total weight of the body, as well as from the qualitative and quantitative composition of the various organs as far as known for the present, we can calculate the carbohydrates of the body (excluding the glycogen), although the results may not be exact, but no doubt are too high or at least are not too low. These calculations of HAMMARSTEN for man and dogs have shown that in the nucleoproteids, glucoproteids, and other substances which are not sugar nor glycogen, but for the sake of brevity are called glucosides, the maximum carbohydrate supply of the body is 5 grams per 1 kilo of body-weight.

If the proteins are to be counted among those bodies which can increase the glycogen of the body, then we must ask the question: Do the proteins act only indirectly as pseudoglycogen-formers or are they direct glycogen-formers which can serve as material for the formation of glycogen or sugar? This question stands in close relationship to the sugar formation and sugar elimination in the various forms of glycosuria and will be discussed best below in connection with the question of diabetes.

Like the carbohydrates in general, glycogen has without any doubt a great importance in the formation of heat and development of energy in the animal body. The possibility of the formation of fat from glycogen cannot be denied.[2] Glycogen is generally considered as reserve food accumulated in the liver and formed in the liver-cells. Where does the glycogen existing in the other organs, such as the muscles, originate? Is the glycogen of the muscles formed on the spot or is it transmitted to the muscles by the blood? These questions cannot yet be answered with positiveness, and the investigations on this subject by different experimenters have given contradictory results. The experiments of KÜLZ,[1] in which he studied the glycogen formation by passing blood containing cane-sugar through the muscle, have led to no conclusive results. Still the formation of glycogen from sugar in the muscles is probable. There is no doubt that glycogen is formed in the muscles during embryonic life.

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[1] Fabian, Zeitschr. f. physiol. Chem., 27; Fränkel and Offer, Centralbl. f. Physiol., 13; Cathcart, Zeitschr. f. physiol. Chem., 39; Bial, Berl. klin. Wochenschr., 1905.

[2] See especially Noël-Paton, Journ. of Physiol., 19.

If it is true that the blood and lymph contain a diastatic enzyme which transforms glycogen into sugar, and also that the glycogen regularly occurs in the form-elements and is not dissolved in the fluids, it seems probable that the glycogen in solution is not transmitted by the blood to the organs, but perhaps more likely, if the leucocytes do not act as carriers, it is formed on the spot from the sugar.[2] The glycogen formation seems to be a general function of the cells. In adults, the liver, which is very rich in cells, has the property, on account of its anatomical position, of transforming large quantities of sugar into glycogen.

The question now arises whether there is any foundation for the statement that the liver-glycogen is transformed into sugar.

As first shown by BERNARD and redemonstrated by many investigators, the glycogen in a dead liver is gradually changed into sugar, and this sugar formation is caused, as BERNARD supposed and ARTHUS and HUBER, PAVY, and recently also PICK and BIAL,[3] proved, by a diastiatic enzyme which, according to RÖHMANN and BORCHARDT,[4] is identical with a diastatic enzyme of the blood.

This post-mortem sugar formation led BERNARD to the assumption of the formation of sugar from glycogen in the liver during life. BERNARD suggested the following arguments for this theory: The liver always contains some sugar under physiological conditions, and the blood from the hepatic vein is always somewhat richer in sugar than the blood from the portal vein. The correctness of either or both of these statements has been disputed by many investigators. PAVY, RITTER, SCHIFF, EULENBERG, LUSSANA, ABELES, and others deny the occurrence of sugar in the liver during life, and the greater amount of dextrose in the blood from the hepatic vein is likewise disputed by them and certain other investigators.[5]

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[1] See Minkowski and Laves, Arch. f. exp. Path. u. Pharm., 23; Külz, Zeitschr. f. Biologie, 27.

[2] See Dastre, Compt. rend. de Soc. biol., 47, 280, and Kaufmann, *ibid.*, 316.

[3] Arthus and Huber, Arch. de Physiol. (5), 4, 659; Pavy, Journal of Physiol., 22; Pick, Hofmeister's Beitr., 3; Bial, Arch. f. (Anat. u.) Physiol., 1901.

[4] Röhmann, Verh. d. Ges. deutsch. Naturf. u. Ärzte, Breslau, 1903; Borchardt, Pflüger's Arch., 100.

[5] In regard to the literature on sugar formation in the liver see Bernard, Leçons sur le diabète, Paris, 1877; Seegen, Die Zuckerbildung im Tierkörper, 2. Aufl., Berlin, 1900; M. Bial, Pflüger's Arch., 55, 434.

It can be said that at present there are two contradictory views on the destruction of the glycogen in the living organism: PAVY'S view, that the glycogen is directly used without being previously transformed into sugar, and BERNARD'S view, which is accepted by most investigators, that the glycogen is first transformed into sugar by the aid of diastatic enzymes. According to certain experimenters (DASTRE, NOËL-PATON, E. CAVAZZANI [1]), who also admit a destruction of the glycogen with the formation of sugar, the change is not brought about by an enzyme, but by a special protoplasmic activity.

The doctrine as to the physiological formation of sugar in the liver has obtained an energetic advocate in SEEGEN. He maintains, after numerous experiments, that the liver regularly contains considerable amounts of sugar. He has observed an increase of 3 per cent in the quantity of dextrose in the liver of a dog kept alive by passing arterial blood through the organ, and lastly he has also found in a very great number of experiments on dogs that the blood from the hepatic vein always contains more—even double as much—sugar than the blood from the portal vein. MOSSE and ZUNTZ [2] have recently made objections as to the correctness of this last statement, and it follows from the various researches on this question that when disturbing influences are prevented, the blood from the hepatic vein is only very little richer in sugar than the blood from the portal vein.

Although SEEGEN energetically espouses the doctrine of BERNARD as to the vital sugar formation in the liver, still he deviates essentially from BERNARD in that he claims the sugar is not derived from the glycogen. According to SEEGEN, the sugar is formed from protein and fat. His older idea, that this protein was peptone, he has discarded. Of importance for the study of the sugar formation in the liver is, on the contrary, the fact that SEEGEN has found a substance in the liver, besides glycogen, which yields dextrose on heating with dilute acids. He, in connection with NEIMANN, has isolated this substance in the form of a nitrogenous carbohydrate. O. SIMON [3] has also recently isolated from the liver a proteose-like substance which reduces directly and yields a fermentable sugar on boiling with acids, and this sugar gives an osazone melting at 190°.

SEEGEN claims to have shown a formation of sugar from fat by a direct experiment with surviving liver tissue. Certain investigations of WEISS seem to substantiate this view, while other experiments of MONTUORI, ABDERHALDEN, and RONA and HESSE contradict this assumption. HILDESHEIM and

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[1] In regard to the literature see Pick, Hofmeister's Beiträge, **3**.

[2] Seegen, Die Zuckerbildung, etc., and Centralbl. f. Physiol., **10**, 497 and 822; Zuntz, *ibid.*, 561; Mosse, Pflüger's Arch., **63**; Bing, Skand. Arch. f. Physiol., **9**.

[3] Seegen, Arch. f. (Anat. u.) Physiol., 1903; Seegen and Neimann, Wien. Sitzungsber., **112** (1903); Simon, Arch. f. exp. Path. u. Pharm., **49**. (See glucothionic acid, page 285.)

LEATHES[1] have made experiments with liver pulp which indicate the reverse, i.e., the formation of fat from glycogen.

The circumstance that the blood-sugar rapidly sinks to $\frac{1}{2}$–$\frac{1}{3}$ of its original quantity, or even disappears when the liver is cut out of the circulation, speaks for a vital formation of sugar in the liver (SEEGEN, BOCK and HOFFMANN; KAUFMANN; TANGL and HARLEY; PAVY). In geese whose livers were removed from the circulation, MINKOWSKI found no sugar in the blood after a few hours. On removing the liver from the circulation by tying all the vessels to and from the organ, the quantity of sugar in the blood on drawing is not increased (SCHENCK[2]). We shall also learn shortly of certain poisons and operative changes which may cause an abundant elimination of sugar, but only when the liver contains glycogen. If we recall the fact shown by RÖHMANN and BIAL[3] that the lymph as well as the blood contains a diastatic enzyme, then several reasons speak for the view of BERNARD that the post-mortem formation of sugar from the glycogen in the liver is a continuation of the vital process.

The relationship of the sugar eliminated in the urine under certain conditions, such as in diabetes mellitus, certain intoxications, lesions of the nervous system, etc., to the glycogen of the liver is also an important question.

It does not enter into the plan and scope of this book to discuss in detail the various views in regard to glycosuria and diabetes. The appearance of dextrose in the urine is a symptom which may have essentially different causes, depending upon different circumstances. Only a few of the most important points will be mentioned.

The blood contains always about an average of 1.5 p. m., while the urine has in it at most only traces of dextrose. When the quantity of sugar in the blood rises to 3 p. m. or above, then sugar passes into the urine. The kidneys have the property to a certain extent of preventing the passage of blood-sugar into the urine; and it follows from this that an elimination of sugar in the urine may be caused partly by a reduction or suppression of this above-mentioned activity, and partly also by an abnormal increase of the quantity of sugar in the blood.

The first seems, according to v. MERING and MINKOWSKI, to be the case in phlorhizin diabetes. v. MERING has found that a strong glycosuria appears in man and animals on the administration of the glucoside phlorhizin. The sugar eliminated is not derived from the glucoside alone. It is formed in the animal body, and in fact, at least on prolonged starvation, from the protein substances of the body. The quantity of sugar in the blood is not increased, but rather diminished, in phlorhizin diabetes (MINKOWSKI), but this is disputed by PAVY. This last investigator found, although only to a slight degree, that the sugar in the blood was increased, but he holds the same view that v. MERING does, that phlorhizin diabetes is a kidney diabetes. That after extirpation of the kidney in phlorhizin diabetics no rise in the blood-sugar is observed, and that after the injection of phlorhizin in the renal artery of one side the urine secreted by this kidney contains sugar sooner and more abundantly than the urine from the other kidney (ZUNTZ), speaks in favor of this view. The experiments especially performed by PAVY, BRODIE, and SIAU[1] upon blood containing phlorhizin and surviving kidneys also indicate the same, namely, that the phlorhizin acts upon the kidneys. While v. MERING believes in an increased permeability of the kidneys for sugar, produced by the phlorhizin, PAVY is, on the contrary, of the opinion that the kidneys, under the influence of the phlorhizin, split off sugar from a substance circulating in the blood, perhaps from a proteid with loosely combined carbohydrate groups.

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[1] Weiss, Zeitschr. f. physiol. Chem., **24**; Montuori, Maly's Jahresb., **26**; Abderhalden and Rona, Zeitschr. f. physiol. Chem., **41**; Hesse, Zeitschr. f. exp. Path. u. Therap., **1**; Hildesheim and Leathes, Journ. of Physiol., **31**.

[2] Seegen, Bock, and Hoffmann, see Seegen, l. c.; Kaufmann, Arch. de Physiol. (5), **8**; Tangl and Harley, Pflüger's Arch., **61**; Pavy, Journ. of Physiol., **29**; Minkowski, Arch. f. exp. Path. u. Pharm., **21**; Schenck, Pflüger's Arch., **57**.

[3] See foot-note 4, p. 295.

With the exception of phlorhizin diabetes, which is dependent, according to the ordinary views, upon a change or special processes in the kidneys, and in which no essential rise in the blood-sugar occurs, all other forms of glycosuria or diabetes, as far as known at present, depend on a *hyperglucæmia.*

A hyperglucæmia may be caused in various ways. It may be caused, for example, by the introduction of more sugar than the body can destroy.

The ability of the animal body to assimilate the different varieties of sugar has naturally a limit. If too much sugar is introduced into the intestinal tract at one time, so that the so-called assimilation limit (see Chapter IX, on absorption) is overreached, then the excess of absorbed sugar passes into the urine. This form of glycosuria is called *alimentary glycosuria*,[2] and it is caused by the passage of more sugar into the blood than the liver and other organs can destroy.

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[1] In regard to the literature on phlorhizin diabetes see v. Mering, Zeitschr. f. klin. Med., **14** and **16**; Minkowski, Arch. f. exp. Path. u. Pharm., **31**; Moritz and Prausnitz, Zeitschr. f. Biologie, **27** and **29**; Külz and Wright, *ibid.*, **27**, 181; Cremer and Ritter, *ibid.*, **28** and **29**; Contejean, Compt. rend. de soc. biol., **48**; Lusk, Zeitschr. f. Biologie, **36**; Levene, Journal of Physiol., **17**; Pavy, *ibid.*, **20**, and with Brodie and Siau, **29**; Arteaga, Amer. Journ. of Physiol., **6**; O. Loewi, Arch. f. exp. Path. u. Pharm., **47**; N. Zuntz, Arch. f. (Anat. u.) Physiol., 1895; Stiles and Lusk, Amer. Journ. of Physiol., **10**; Cremer, Ergebnisse der Physiol., **1**, Abt. 1, and the monographs upon diabetes.

[2] In regard to alimentary glycosuria see Moritz, Arch. f. klin. Med., **46**, which also contains the older literature; B. Rosenberg, Ueber das Vorkommen der alimentären Glykosurie, etc. (Inaug.-Dissert. Berlin, 1897); van Oondt, Münch. med. Wochenschr., 1898; v. Noorden, Die Zuckerkrankheit, 3. Aufl., 1901.

As the liver cannot transform into glycogen all the sugar which comes to it in alimentary glycosuria, it is possible that a glycosuria may be produced also under pathological conditions, even by a moderate amount of carbohydrate (100 grams dextrose), which a healthy person could overcome. This is the case, among others, in various affections of the cerebral system and in certain chronic poisonings. Certain observers include the lighter forms of diabetes in this class of glycosuria.

We differentiate between light and severe forms of diabetes. In the first the urine contains sugar only when carbohydrates are taken as food, while in the other case the urine contains sugar even with food entirely free from carbohydrates. According to the views of several investigators, in light forms of diabetes the liver is incapable of transforming into glycogen all the carbohydrates introduced, or to utilize this glycogen in a normal way, and the activity of the liver-cells is also reduced or changed in these cases.

A hyperglucæmia which passes into a glycosuria may also be brought about by an excessive formation of sugar from the glycogen and other substances within the animal body.

The so-called *piqûre*, and also probably those glycosurias which occur after other lesions of the nervous system, belong to the above group of glycosurias. The glycosuria produced on poisoning with carbon monoxide, adrenalin, curare, strychnine, morphine, etc., also belongs to this group. That the glycosuria produced in certain cases, as after *piqûre*, is due to an increased transformation of the glycogen follows from the fact that no glycosuria appears, under the above-mentioned circumstances, when the liver has been previously made free from glycogen by starvation or other means. In other cases, as in carbon-monoxide poisoning, the sugar is probably derived from the proteins, because glucosuria occurs only in those cases where the poisoned animal has a sufficient quantity of protein at its disposal (STRAUB and ROSENSTEIN[1]). Protein starvation with a simultaneously abundant supply of carbohydrates causes this glycosuria to disappear.

A hyperglucæmia with glycosuria may also be caused by a decreased ability of the animal body to consume or destroy the sugar. In this case the sugar must accumulate in the blood, and the formation of severe cases of diabetes mellitus is now generally explained by this process.

The inability of diabetics to destroy or consume the sugar does not seem to be connected with any decrease in the oxidative energy of the cells. The oxidative processes are not diminished generally in diabetics

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[1] See Dock, Pflüger's Arch., 5; Bock and Hoffmann, Exp. Studien über Diabetes (Berlin, 1874); Cl. Bernard, Leçons sur le diabète (Paris); T. Araki, Zeitschr. f. physiol. Chem., 15, 351; Straub, Arch. f. exp. Path. u. Pharm., 38; Rosenstein, *ibid.*, 40; Pflüger, Pflüger's Arch., 96.

(SCHULTZEN, NENCKI and SIEBER[1]), and this has recently been substantiated by BAUMGARTEN. This latter investigator made experiments with several bodies which on account of their aldehyde nature were closely related to sugar or were cleavage or oxidation products of the same, namely, glucuronic acid, *d*-gluconic acid, *d*-saccharic acid, glucosamine, mucic acid, and others, and he found that diabetics destroyed or burnt these bodies to the same extent as healthy individuals. Besides this it must be remarked that the two varieties of sugar, dextrose and levulose, which are oxidized with the same readiness, act differently in diabetics. According to KÜLZ and other investigators levulose is, contrary to dextrose, utilized to a great extent in the organism, and may, according to MINKOWSKI,[2] even cause a deposit of glycogen in the liver in animals with pancreas diabetes (see below). The combustion of protein and fat takes place as in healthy subjects, and the fat is completely burned into carbon dioxide and water. In this diabetes the ability of the cells to utilize especially the dextrose suffers diminution, and the explanation of this has been sought in the fact that the dextrose is not previously split before combustion.

The variation in the respiratory quotient, i.e., the relation $\frac{CO_2}{O}$, seems to show an insufficiency of the dextrose combustion in the tissues in diabetes. As will be thoroughly explained in a following chapter, this quotient is greater the more carbohydrates are burnt in the body, and it is correspondingly smaller when protein and fat are chiefly burnt. The investigations of LEO, HANRIOT, WEINTRAUD and LAVES,[3] and others have shown that in severe cases of diabetes, in the starving condition the low quotient is not raised after partaking of dextrose, as in healthy individuals, but that it is raised after feeding levulose, which is also of value to diabetics (WEINTRAUD and LAVES). The poverty of the organs and tissues of diabetics in glycogen shows that not only is the combustion of the dextrose diminished, but also the transformation of the same into glycogen, and its valuation as a whole is decreased.

There are also certain investigators who consider that diabetes is due to an increased production of sugar in the liver—a view which has received some support in the artificially produced pancreatic diabetes.

The investigations of MINKOWSKI, v. MERING, DOMENICIS, and later of many other investigators[1] have shown that a true diabetes of a severe kind is caused by the total or nearly total extirpation of the pancreas of many animals, especially dogs. As in man in severe forms of diabetes, so also in dogs with pancreatic diabetes, an abundant elimination of sugar takes place even on the complete exclusion of carbohydrates from the food.

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[1] Schultzen, Berl. klin. Wochenschr., 1872; Nencki and Sieber, Journ. f. prakt. Chem. (N. F.), **26**, 35; Baumgarten, "Ein Beitrag zur Kenntniss des Diabetes mellitus," Habilitationsschrift, also Zeitschr. f. exp. Path. u. Therap., **2**, 1905.

[2] Külz, Beiträge zur Path. u. Therap. des Diabetes mellitus (Marburg, 1874), **1**; Weintraud and Laves, Zeitschr. f. physiol. Chem., **19**; Haycraft, *ibid.*; Minkowski, Arch. f. exp. Path. u. Pharm., **31**.

[3] See v. Noorden, Die Zuckerkrankheit, 3. Aufl., 1901.

Artificial pancreas diabetes may, at least in cases where the pancreas has not been completely extirpated, present exactly the same conditions as diabetes in man, but opinions differ as to the cause of this diabetes. It is generally accepted that in pancreas diabetes a diminished consumption of sugar takes place; but there are several investigators who are of another opinion and who explain this form of diabetes as due at least not entirely to a diminished combustion of sugar, but to a diseased increase in the sugar formation. From this it follows that the pancreatic gland exerts on the formation of sugar in the liver a regulating action which is absent on the extirpation of the gland.

Many important observations show that a close relation exists between the liver and pancreas diabetes. **Pflüger** has also shown that especially in diabetes produced by **Sandmeyer's** method (partial extirpation with subsequent destruction of the remains of the gland in the abdominal cavity, when the animal remains alive for a longer time than after total extirpation) the liver does not lose weight, although the total weight of the animal diminishes greatly, while in starvation without diabetes the liver loses weight more than the other parts of the body. **Pflüger** concludes from this that the liver in diabetes works actively and is the most important seat of production of diabetic sugar.

We do not know how the pancreas acts in the formation or the destruction of sugar, and we have essentially two contradictory views on this subject. According to one view the action is of a nervous kind, while the other view is that we are dealing with an internal secretion of special bodies which in an unknown manner perhaps act upon the nerve centres and regulate the formation or the destruction of sugar. The assumption of an internal secretion is rather generally accepted and is based on the investigations of **Minkowski**, **Hédon**, **Lanceraux**, **Thiroloix**, and others[2] upon the action of the subcutaneous transplantation of the gland. According to these investigations a subcutaneously transplanted piece of the gland can completely perform the functions of the pancreas as to the sugar exchange and the sugar elimination, because on the removal of the intra-abdominal piece of gland the animal in this case does not become diabetic. But if the subcutaneously embedded piece of pancreas is then subsequently removed, an active elimination of sugar appears immediately. Pflüger has made important objections to the force of proof in these experiments.

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[1] See Minkowski, Untersuchungen über Diabetes mellitus nach Exstirpation des Pankreas (Leipzig, 1893); v. Noorden, Die Zuckerkrankheit (Berlin, 1901), which contains a very copious index of the literature. In regard to diabetes see also Cl. Bernard, Leçons sur le diabète (Paris); Seegen, Die Zuckerbildung im Thierkörper (Berlin, 1890), and Pflüger, Das Glykogen, 2. Aufl., 1905.

[2] See Minkowski, Arch. f. exp. Path. u. Pharm., 31; Hédon, Diabète pancréatique, Travaux de Physiologie (Laboratoire de Montpellier, 1898), and the works on diabetes.

This internal secretion of the pancreas has in recent times been supposed to be connected with the so-called islands of Langerhans; but no positive results have been obtained in this connection.[1] We are also not acquainted with the kind of active substance here formed.

The glycolytic property of the blood as shown by Lépine was considered for a time to be due to a glycolytic enzyme formed in the pancreas, and pancreas diabetes used to be explained by the fact that the action of this enzyme was removed when the gland was extirpated. This glycolysis is not sufficient, even if it is derived from the pancreas, to explain the transformation of the large quantity of sugar in the body, and for the destruction of sugar we are also obliged to accept a glycolysis in the organs and tissues. The views in regard to this glycolysis differ in certain points. According to one view (Spitzer and others) special oxidases are active in the glycolysis, while another view (Stoklasa) considers the glycolysis as analogous to alcoholic fermentation, where we have processes brought on by special tissue zymases (see Chapter I).

Another important question is whether one organ can bring about glycolysis or whether a combination of organs is required. Cohnheim has found that a cell-free fluid can be obtained from a mixture of pancreas and muscle, which destroys dextrose, while the pancreas alone does not have this action and the muscle only to a slight extent. The pancreas does not contain, according to Cohnheim, a glycolytic enzyme, but a substance resistant to boiling temperatures, which is soluble in water and alcohol, and which, like an amboceptor, activates a glycolytic proenzyme which exists in the muscle fluid, but which is inactive alone and which retards glycolysis when it exists in excess. De Meyer holds a nearly similar view, but with this exception, that he does not consider that the activating substance comes from the muscles but from the leucocytes. Lépine[2] has also expressed the opinion that the pancreas does not have a direct glycolytic action by internal secretion, but more likely by the glycolysis encouraged by the action of cell protoplasm.

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[1] See Diamare and Kubiabko, Centralbl. f. Physiol., **18**, and Diamare, *ibid.*, **19**, Rennie, *ibid.*, **18**; Sauerbeck, Virchow's Arch., **177**.

[2] Cohnheim, Zeitschr. f. physiol. Chem., **39**, **42**, **43**, and **47**; De Meyer, Arch intern. de Physiol., **2**, cited from Biochem. Centralbl., **3**.

The statements of **Cohnheim** have not been fully confirmed by other investigators. On the contrary, several investigators, **Stoklasa** and collaborators, **Feinschmidt**, **Arnheim** and **Rosenbaum**, and **Braunstein**,[1] could not detect any glycolytic activity either in the pancreas alone or in muscles and other organs alone (with the exclusion of bacteria). The liver also belongs to these organs, in which, it must be remarked, the glycolytically active substance has been absent in severe cases of diabetes. **Cohnheim's** statements have, on the contrary, been substantiated in part by **Arnheim** and **Rosenbaum** and **R. Hirsch**, who find that the pancreas has the power of raising the glycolytic action of the liver and the muscles. On the other hand, **Claus** and **Embden** have not been able to obtain the activating action of the pancreas upon muscle-juice, but according to **Cohnheim** this is probably due to the fact that these investigators added too large quantities of pancreas, whereby the retarding action came into effect. No positive conclusions on the mode of action of the pancreas in sugar destruction or sugar formation can be drawn from these contradictory statements.

Where does the sugar eliminated in diabetes originate? Does it depend entirely upon the carbohydrates of the food or the store of carbohydrate in the body, or has the body the power of producing sugar from other material? To **Lüthje** belongs the credit for positively deciding this question. He has made experiments on dogs with pancreas diabetes, in which on a protein diet free from carbohydrates so much sugar was eliminated that it could not possibly be accounted for by the store of glycogen or other carbohydrate-containing substances in the body. Similar experiments have also been performed later by **Pflüger**,[2] and the power of the animal body to produce sugar from non-carbohydrate material is now definitely proven.

Is this sugar produced from protein or fat, or from both? This question so far has not been answered, and it is the subject of continuous dispute. It is not possible to enter into an exhaustive and detailed discussion of the question in a text-book, and we will only mention briefly certain of the most important observations and historical points.

The largest amount of sugar which we can obtain theoretically from protein is 8 grams of sugar from 1 gram of protein nitrogen if we admit that all the carbon of the protein, with the exception of that necessary to form ammonium carbonate, is used for the formation of sugar. The actual relation between dextrose and nitrogen in the urine, i.e., the quotient D:N, has been repeatedly determined in various forms of diabetes. In a large number of cases this has been found to be equal to 2.8 to 3.8. It may undergo considerable variation, and in certain cases it may indeed be lower than 1 as well as higher than 8. From these quotients conclusions have been drawn as to the amount of sugar formed, as well as the origin of the sugar, but according to the views of HAMMARSTEN such conclusions are mostly very uncertain. The sugar eliminated by the urine represents the difference between the total sugar production of the body and the quantity of sugar burned or utilized. Only under the supposition that the body cannot burn or utilize any sugar is the sugar of the urine a measure of the quantity of sugar produced; it is not known how far this supposition can be applied in the various forms of diabetes. Still several observations seem to show that in the different forms of diabetes variable amounts of the sugar are burned. A sugar formation from fat can be presumed only when the quotient is specially high.

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[1] Stoklasa and collaborators, Centralbl. f. Physiol., 17, and Ber. d. d. chem. Gesellsch., 36 and 38; Feinschmidt, Hofmeister's Beiträge, 4; Hirsch, *ibid.*; Claus and Embden, *ibid.*, 6; Arnheim and Rosenbaum, Zeitschr. f. physiol. Chem., 40; Braunstein, Zeitschr. f. klin. Med., 51.

[2] Lüthje, Deutsch. Arch. f. klin. Med., 79, and Pflüger's Arch., 106; Pflüger, Pflüger's Arch. 108

The property of protein of increasing the elimination of sugar is considered as an important proof of the formation of sugar from protein. In this regard those experiments are of special interest in which the diabetic animal is allowed to starve until the urine is poor in sugar or indeed free from sugar, and then by feeding with protein an abundant elimination of sugar is produced. If we do not want to accept in this case that the protein, but rather the fat, was the material from which the sugar was produced, still we must admit either of a sugar-sparing action due to protein or of a strong sugar formation from fat, incited by the protein.

A sparing in the sense that the protein is oxidized instead of the sugar, and in this manner protects it, is naturally possible only under the supposition that the body can burn at least a part of the sugar, otherwise there would be nothing to spare and nothing to protect from burning. The assumption of such an indirect action of proteins is difficult to reconcile with the common view of the inability of the body to burn sugar in diabetes. LÜTHJE[1] has communicated one experiment among others, in which a dog with pancreas diabetes, whose weight before starvation was 18 kilos, with nineteen days' starvation eliminated an average of 10.4 grams sugar for the last six days of starvation. By exclusive protein feeding the quantity of sugar per day could be raised to a maximum of 123.8 grams, and as average it was 97.5 grams for the ten protein days. The protein therefore had protected daily an average of 87 grams sugar from burning, which is hardly possible; and if in the diabetic animal we admit of this considerable power of burning sugar the quotient D:N becomes valueless as a measure of the quantity of sugar formed.

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[1] Deutsch. Arch. f. klin. Med., 79.

If, on the contrary, we admit of an indirect action of proteins in that they incite a sugar formation from fat, perhaps by a certain very important increase in the activity of the liver, we are opposed by the great difficulty that, according to known laws of metabolism, the proteins do not raise the fat metabolism, but rather diminish it. The protein displaces a corresponding quantity of fat from the metabolism, and if the fat was the only source of sugar then in this case we would expect a diminished elimination of sugar instead of an increased one. Nevertheless the above action of protein upon sugar elimination is much more easily explained by the assumption of a sugar formation from protein than from fat.

The action of monamino-acids upon the carbohydrate metabolism has also given important ground for the assumption of a sugar formation from protein. That a deamidation occurs in the animal body was shown by the older observations of BAUMANN and BLENDERMANN. Further proofs of this were furnished by the recent investigations of NEUBERG and LANGSTEIN, where in feeding experiments with alanine they found abundance of lactic acid in urine, and finally LANG[1] has shown that various organs in antiseptic autolysis have the power of deamidating amides and amino-acids. As from amino-acids by deamidation it is possible to produce oxyfatty acids according to the formula $\text{—CH.NH}_2+\text{H}_2\text{O}=\text{—CH(OH)}+\text{NH}_3$, it was interesting to test the action of amino-acids upon carbohydrate metabolism. Several investigations have been carried on with this in view, such as those of LANGSTEIN and NEUBERG, R. COHN and F. KRAUS, which have shown a very probable formation of carbohydrate under the influence of amino-acids; but the investigations of EMBDEN and SALOMON and of EMBDEN and ALMAGIA[2] have positively shown in a dog without a pancreas that the amino-acids can bring about a re-formation of carbohydrate. It is still an open question whether the amino-acids are only indirectly active in this or whether they form the material from which the sugar is formed. In general we consider the formation of sugar with amino-acids as intermediary bodies as very probable.

If we presume a formation of sugar from fat we must differentiate between the two components of neutral fats, that is, between the glycerine and the fatty acids. A formation of sugar from glycerine can be considered as proven from the investigations of CREMER, and especially those of LÜTHJE,[3] and in what follows we will discuss only the formation of sugar from the fatty acids.

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[1] Baumann, Zeitschr. f. physiol. Chem., 4; Blendermann, *ibid.*, 6; Neuberg and Langstein, Arch. f. (Anat. u.) Physiol., 1903, Suppl.; Lang, Hofmeister's Beiträge, 5.

[2] Langstein and Neuberg, l. c.; Cohn, Zeitschr. f. physiol. Chem., 28; F. Kraus, Berl. klin. Wochenschr., 1904; Embden and Salomon, Hofmeister's Beiträge, 5 and 6, and with Almagia, *ibid.*, 7.

[3] Cremer, Sitzungsber. d. Ges. f. Morph. u. Physiol. München, 1902; Lüthje, Deutsch. Arch. f. klin. Med., 80.

The formation of sugar from fat seems to occur in the plant kingdom, and as the chemical processes in the animal and plant life are in principle the same it makes the possibility of a sugar formation from fat very probable. Such an origin of sugar in the animal body is accepted by many investigators, especially by PFLÜGER and several French observers, among whom we must specially mention CHAUVEAU and KAUFMANN.[1]

Where food as free from carbohydrate as possible is taken, the quotient D: N is high, i.e., higher than 8, as well as when the quantity of sugar is so large that it cannot be accounted for by the calculated protein (and carbohydrate) metabolism, then if the observations are otherwise free from error we can admit of a formation of sugar from fat. Several such cases of diabetes in man have been published (RUMPF, ROSENQVIST, MOHR, v. NOORDEN, and others), and also in animals (HARTOGH and SCHUMM).[2] Although these researches are not fully conclusive, still certain of them indicate a probable formation of sugar from fat. We also have several conditions which indicate the same, namely, that in phlorhizin diabetes after the disappearance of the liver-glycogen the fat which migrates to the liver serves as material for the formation of sugar (PFLÜGER); still this is not sufficient as a positive proof.

Starting with the quotient D:N, which he sets at 3.67, MAGNUS-LEVY has calculated the quantity of oxygen necessary for the combustion of the protein, provided the sugar was formed therefrom, and also the quantity of carbon dioxide produced, i.e., the respiratory quotient for these cases. On comparison of these results with the low respiratory quotient observed in diabetics, he comes to the conclusion that the sugar is derived from the protein. PFLÜGER,[3] who has made a different calculation, comes to an entirely different result, and considers that the low values for the respiratory quotient in diabetes are positive proof that the sugar is not formed from the proteins, but from the fats. As the quotient D:N is not an accurate measure of the quantity of sugar formed, and as we cannot, for the present, exactly measure the quantity of oxygen necessary for the formation of sugar from the protein, HAMMARSTEN believes that it is just as impossible to conclude from the respiratory quotient that sugar is formed from the fats as from the proteids.

We have no exact proofs of a sugar formation from fat or from protein alone, nevertheless we have proofs of the possibility of a formation from both of these. There is really no objection to the assumption that the body has the power of producing sugar from protein as well as from fat. The observations on the formation of sugar or on the carbohydrate metabolism in diabetes do not give any positive explanations as to the question whether proteins are direct glycogen-formers or not.

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[1] Kaufmann, Arch. f. Physiol. (5), 8, where Chauveau's work is cited.

[2] Rumpf, Berl. klin. Wochenschr., 1899; Rosenqvist, *ibid.*; Mohr, *ibid.*, 1901; v. Noorden, Die Zuckerkrankheit, 3. Aufl., Berlin, 1901; Hartogh and Schumm, **Arch. f.** Path. u. Pharm., **45**. See also the works of O. Loewi, *ibid.*, **47**, and Lusk, **Zeitschr. f.** Biologie, **42**.

[3] Magnus-Levy, Zeitschr. f. klin. Med., 56; Pflüger, Pflüger's Arch., **108**.

The Bile and its Formation.

By the establishment of a biliary fistula, an operation which was first performed by SCHWANN in 1844 and which has been improved lately by DASTRE and PAWLOW,[1] it is possible to study the secretion of the bile. This secretion is continuous, but with varying intensity. It takes place under a very low pressure; therefore an apparently unimportant hindrance in the outflow of the bile, namely, a stoppage of mucus in the exit, or the secretion of large quantities of viscous bile, may cause stagnation and absorption of the bile by means of the lymphatic vessels (absorption icterus).

The quantity of bile secreted in the twenty-four hours in dogs can be exactly determined. The quantity secreted by different animals varies, and the limits are 2.9–36.4 grams of bile per kilo of weight in the twenty-four hours.[2]

The statements as to the extent of bile secretion in man are few and not to be depended on. RANKE found (using a method which is not free from criticism) a secretion of 14 grams of bile with 0.44 gram of solids per kilo in twenty-four hours. NOËL-PATON, MAYO-ROBSON, HAMMARSTEN, PFAFF and BALCH, and BRAND[3] have found a variation between 514 and 1083 c.c. per twenty-four hours. Such determinations are of doubtful value, because in most cases it follows from the composition of the collected bile that the fluid is not the result of a secretion of normal liver bile.

The quantity of bile secreted is, however, as specially shown by STADELMANN,[4] subject to such great variation even under physiological conditions that the study of those circumstances which influence the secretion is very difficult and uncertain. The contradictory statements by different investigators may probably be explained by this fact.

In starvation the secretion diminishes. According to LUKJANOW and

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[1] Schwann, Arch. f. (Anat. u.) Physiol., 1844; Dastre, Arch. de Physiol. (5), 2; Pawlow, Ergebnisse der Physiol., 1, Abt. 1.

[2] In regard to the quantity of bile secreted in animals see Heidenhain, Die Gallenabsonderung, in Hermann's Handbuch der Physiol., 5, and Stadelmann, Der Icterus und seine verschiedenen Formen (Stuttgart, 1891).

[3] Ranke, Die Blutvertheilung und der Thätigkeitswechsel der Organe (Leipzig, 1871); Noël-Paton, Rep. Lab. Roy. Coll. Edinburgh, 3; Mayo-Robson, Proc. Roy. Soc., 47; Hammarsten, Nova Act. Reg. Soc. Scient. Upsala (3), 16; Pfaff and Balch, Journ. of Exp. Med., 1897; Brand, Pflüger's Arch., 90.

[4] Stadelmann, Der Icterus, etc., Stuttgart, 1891.

ALBERTONI,[1] under these conditions the absolute quantity of solids decreases, while the relative quantity increases. After partaking of food the secretion increases again. The statements are very contradictory in regard to the time necessary after partaking of food before the secretion reaches its maximum. After a careful examination and compilation of all the existing statements HEIDENHAIN [2] has come to the conclusion that in dogs the curve of rapidity of secretion shows two maxima, the first at the third to fifth hour and the second at the thirteenth to fifteenth hour after partaking of food. According to BARBÉRA the time when the maximum occurs is dependent upon the kind of food. With carbohydrate food it is two to three hours, after protein food three to four hours, and with fat diet it is five to seven hours after feeding.

According to the older statements, the proteins, of all the various foods, cause the greatest secretion of bile, while the carbohydrates diminish the secretion, or at least excite it much less than the proteins. This coincides with the recent observations of BARBÉRA.[3] The authorities are by no means ageed as to the action of the fats. While many older investigators have not observed any increase, but rather the reverse, in the secretion of bile after feeding with fats, the researches of BARBÉRA show an undoubted increase in the secretion of bile on fat feeding, greater even than after carbohydrate feeding. According to ROSENBERG olive-oil is a strong cholagogue, a statement which, according to other investigators—MANDELSTAMM, DOYON and DUFOURT [4]—is not sufficiently proved.

As BARBÉRA has shown, a close relationship exists between the bile secretion and the quantity of urea formed, as an increase in the first goes hand in hand with an increase of the latter. The bile is, therefore, according to him, a product of disassimilation, whose quantity rises and falls with the degree of activity of the liver.

The question whether there exist special medicinal bodies, so-called cholagogues, which have a specific excitant action on the secretion of bile has been answered in very different ways. Many, especially the older investigators, have observed an increase in the bile secretion after the use of certain therapeutic agents, such as calomel, rhubarb, jalap, turpentine, olive-oil, etc.; while others, especially the more recent investigators, have arrived at quite opposite results. From all appearances this contradiction is due to the great irregularity of the normal secretion, which might readily cause mistakes in tests with therapeutic agents.

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[1] Lukjanow, Zeitschr. f. physiol. Chem., **16**; Albertoni, Recherches sur la sécrétion biliaire, Turin, 1893.

[2] Hermann's Handb., **5**, and Stadelmann, Der Icterus, etc.

[3] Centralbl. f. Physiol., **12** and **16**.

[4] Barbéra, Bull. della scienz. med. di Bologna (7), **5**, Maly's Jahresber., **24**, and Centralbl. f. Physiol., **12** and **16**; Rosenberg, Pflüger's Arch., **46**; Mandelstamm, Ueber den Einfluss einiger Arzneimittel auf Sekretion und Zusammensetzung der Galle (Dissert. Dorpat, 1890); Doyon and Dufourt, Arch. de Physiol. (5), **9**. In regard to the action of various foods on the secretion of bile see also Heidenhain, l. c.; Stadelmann, Der Icterus; and Barbéra, l. c.

SCHIFF's view, that the bile absorbed from the intestinal canal increases the secretion of bile and hence acts as a cholagogue, seems to be a positively proven fact by the investigations of several experimenters.[1] Sodium salicylate is also perhaps a cholagogue (STADELMANN, DOYON and DUFOURT).

Acids, and especially, under normal conditions, hydrochloric acid, seem to be physiological excitants for bile secretion. According to FALLOISE and FLEIG the acids act upon the duodenum and the upper part of the jejunum, and the action is brought about by a secretin formation similar to the action of acids upon the secretion of pancreatic juice (see Chapter IX). According to FALLOISE[2] chloral hydrate introduced into the duodenum causes a secretion of bile in an analogous manner by the aid of a special *chloral secretin.*

The bile is a mixture of the secretion of the liver-cells and the so-called mucus which is secreted by the glands of the biliary passages and by the mucous membrane of the gall-bladder. The secretion of the liver, which is generally poorer in solids than the bile from the gall-bladder, is thin and clear, while the bile collected in the gall-bladder is more ropy and viscous on account of the absorption of water and the admixture of "mucus," and cloudy because of the admixture of cells, pigments, and the like. The specific gravity of the bile from the gall-bladder varies considerably, being in man between 1.010 and 1.040. Its reaction is alkaline to litmus. The color changes in different animals: golden yellow, yellowish brown, olive-brown, brownish green, grass-green, or bluish green. Bile obtained from an executed person immediately after death is golden yellow or yellow with a shade of brown. Still cases occur in which fresh human bile from the gall-bladder has a green color. The ordinary post-mortem bile has a variable color. The bile of certain animals has a peculiar odor; for example, ox-bile has an odor of musk, especially on warming. The taste of bile is also different in different animals. Human as well as ox bile has a bitter taste, with a sweetish after-taste. The bile of the pig and rabbit has an intensely persistent bitter taste. On heating bile to boiling it does not coagulate. It contains (in the ox) only traces of true mucin, and its ropy properties depend, it seems, chiefly on the presence of a nucleoalbumin similar to mucin (Paijkull). The bile from the animals investigated by Hammarsten showed a similar behavior. Hammarsten[1] has, on the contrary, found a true mucin in human bile. To all appearances this mucin originates from the biliary passages, as he found it in the bile flowing from the hepatic duct, and also because the mucous membrane of the gall-bladder, according to Wahlgren,[2] does not in man secret any mucin, but a mucin-like nucleoalbumin.

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[1] Schiff, Pflüger's Arch., 3. See Stadelmann, Der Icterus, and the dissertations of his pupils, especially Winteler, "Experimentelle Beiträge zur Frage des Kreislaufes der Galle" (Inaug.-Diss. Dorpat, 1892), and Gärtner, "Experimentelle Beiträge zur Physiol. und Path. der Gallensekretion" (Inaug.-Diss. Jurjew, 1893); also Stadelmann, "Ueber den Kreislauf der Galle," Zeitschr. f. Biologie, 34.

[2] Falloise, Bull. Acad. Roy. de Belg., 1903; Fleig, *ibid.*, 1903.

The specific constituents of the bile are *bile-acids* combined with alkalies, *bile-pigments*, and, besides small quantities of *lecithin* and *phosphatides*, *cholesterin*, *soaps*, *neutral fats*, *urea*, *ethereal sulphuric acid*, traces of *conjugated glucuronic acids* and *mineral substances*, chiefly chlorides, besides phosphates of calcium, magnesium, and iron. Traces of copper also occur.

Bile-salts. The bile-acids which thus far have best been studied may be divided into two groups, the *glycocholic* and *taurocholic acid* groups. As found by Hammarsten,[3] a third group of bile-acids occurs in the shark and probably also in other animals. These are rich in sulphur, and like the ethereal sulphuric acids they split off sulphuric acid on boiling with hydrochloric acid. All glycocholic acids contain nitrogen, but are free from sulphur and can be split with the addition of water into glycocoll (amino-acetic acid) and a nitrogen-free acid, a cholic acid. All taurocholic acids contain nitrogen and sulphur and are split, with the addition of water, into taurine (aminoethylsulphonic acid) and a cholic acid. The reason for the existence of different glycocholic and taurocholic acids depends on the fact that there are several cholic acids.

The conjugated bile-acid found in the shark, and called *scymnol sulphuric acid* by Hammarsten, yields as cleavage products sulphuric acid and a non-nitrogenous substance, *scymnol* ($C_{27}H_{46}O_5$), which gives the characteristic color reactions of cholic acid.

The different bile-acids occur in the bile as alkali salts, generally the sodium compounds, even in sea-fishes, although this is contrary to the older statements (Zanetti[4]). In the bile of certain animals we find almost solely glycocholic acid, in others only taurocholic acid, and in other animals a mixture of both (see further on).

All alkali salts of the biliary acids are soluble in water and alcohol, but insoluble in ether. Their solution in alcohol is therefore precipitated by ether, and this precipitate, with proper care in manipulation, gives, for nearly all kinds of bile thus far investigated, rosettes or balls of fine needles or four- to six-sided prisms (PLATTNER's crystallized bile). Fresh human bile also crystallizes readily. The bile-acids and their salts are optically active and dextrorotatory. The salts of the different bile-acids act somewhat differently towards neutral salts. The alkali salts of the ordinary and best-studied bile-acids from man, ox, and dog are, according to TENGSTRÖM,[1] precipitated by ammonium and magnesium sulphates, and also, in pure form, by sodium nitrate and sodium chloride (added to saturation). Potassium and sodium sulphates do not precipitate them. The alkali salts cannot be directly precipitated from the bile by NaCl, on account of the presence of bodies retarding precipitation, among which we find oil-soaps.

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[1] Paijkull, Zeitschr. f. physiol. Chem., **12**; Hammarsten, l. c., Nova Act. (3), **16**, and Ergebnisse der Physiol., Bd. **4**.

[2] Maly's Jahresber., **32**.

[3] Hammarsten, Zeitschr. f. physiol. Chem., **24**.

[4] See Chem. Centralbl., 1903, **1**, 180.

The bile-acids are dissolved by concentrated sulphuric acid at the ordinary temperature, forming a reddish-yellow liquid which has a beautiful green fluorescence. According to PREGL an oxidation with reduction of the sulphuric acid into sulphur dioxide takes place. The fluorescent substance has been called dehydrocholan (see below) by PREGL.[2] On carefully warming with concentrated sulphuric acid and a little cane-sugar, the bile-acids give a beautiful cherry-red or reddish-violet liquid. PETTENKOFER's reaction for bile-acids is based on this behavior.

PETTENKOFER's *test for bile-acids* is performed as follows: A small quantity of bile in substance is dissolved in a small porcelain dish in concentrated sulphuric acid and warmed, or some of the liquid containing the bile-acids is mixed with concentrated sulphuric acid, taking special care in both cases that the temperature does not rise higher than 60–70° C. Then a 10 per cent solution of cane-sugar is added, drop by drop, continually stirring with a glass rod. The presence of bile is indicated by the production of a beautiful red liquid, whose color does not disappear at the ordinary temperature, but becomes more bluish violet in the course of a day. This red liquid shows a spectrum with two absorption-bands, the one at F and the other between D and E, near E.

This extremely delicate test fails, however, when the solution is heated too high or if an improper quantity—generally too much—of the sugar is added. In the last-mentioned case the sugar easily carbonizes and the test becomes brown or dark brown. The reaction fails if the sulphuric acid contains sulphurous acid or the lower oxides of nitrogen. Many other substances, such as proteins, oleic acid, amyl alcohol, and morphine, give a similar reaction, and therefore in doubtful cases the spectroscopic examination of the red solution must not be forgotten.

PETTENKOFER's test for the bile-acids depends essentially on the fact that furfurol is formed from the sugar by the sulphuric acid, and this body can therefore be substituted for the sugar in this test (MYLIUS). According to Mylius and v. Udranszky[1] a 1 p. m. solution of furfurol should be used. Dissolve the bile, which must first be purified by animal charcoal, in alcohol. To each cubic centimeter of alcoholic solution of bile in a test-tube add 1 drop of the furfurol solution and 1 c.c. concentrated sulphuric acid, and cool when necessary, so that the test does not become too warm. This reaction, when performed as described, will detect $\frac{1}{20}$ to $\frac{1}{30}$ milligram cholic acid (v. Udranszky). Other modifications of Pettenkofer's test have been proposed.

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[1] Zeitschr. f. physiol. Chem., 41.

[2] Zeitschr. f. physiol. Chem., 45.

Glycocholic Acid. The constitution of the glycocholic acid occurring in human and ox bile, and which has been most studied, is represented by the formula $C_{26}H_{43}NO_6$. Glycocholic acid is absent, or nearly so, in the bile of carnivora. On boiling with acids or alkalies this acid, which is analogous to hippuric acid, is converted into cholic acid and glycocoll.

By the action of hydrazine hydrate upon the ethyl ester of cholic acid Bondi and Müller[2] have prepared first cholic-acid hydrazide, and then, by the action of nitrous acid upon this, they obtained the cholic-acid azide, $C_{23}H_{39}O_3CO.N_3$, and finally from this last in alkaline solution with glycocoll they synthetically prepared the alkali salt of glycocholic acid, at the same time splitting off nitrogen.

Glycocholic acid crystallizes in fine, colorless needles or prisms. It is soluble with difficulty in water (in about 300 parts cold and 120 parts boiling water), and is easily precipitated from its alkali-salt solution by the addition of dilute mineral acids. It is readily soluble in strong alcohol, but with great difficulty in ether. The solutions have a bitter but at the same time sweetish taste. The acid melts at 138–140° C. (Medvedew[3]). The salts of the alkalies and alkaline earths are soluble in alcohol and water.

The solution of the alkali salt in water can be salted out by NaCl, but not by KCl. The salts of the heavy metals are mostly insoluble or soluble with difficulty in water. The solution of the alkali salts in water is precipitated by sugar of lead, cupric and ferric salts, and silver nitrate.

Glycocholeic Acid is a second glycocholic acid, first isolated by Wahlgren[4] from ox-bile, and has the formula $C_{26}H_{43}NO_5$ or $C_{27}H_{45}NO_5$. This acid, which on hydrolytic cleavage yields glycocoll and choleic acid, has also been detected in human bile and the bile of the musk-ox (Hammarsten[5]).

Glycocholeic acid may, like glycocholic acid, crystallize in tufts of fine needles, but is often obtained as short thick prisms. It is much more insoluble in water, even on boiling, than glycocholic acid, and it melts at

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[1] Mylius, Zeitschr. f. physiol. Chem., **11**; v. Udranszky, *ibid.*, **12**.

[2] Zeitschr. f. physiol. Chem., **47**.

[3] Centralbl. f. Physiol., **14**.

[4] Zeitschr. f. physiol. Chem., **36**.

[5] *Ibid.*, **43**.

175–176° C. The alkali salts are soluble in water, have a pure bitter taste, and are more readily precipitated by neutral salts (NaCl) than the glycocholates. The solution of the alkali salts is not only precipitated by the salts of the heavy metals, but also by the salts of barium, calcium and magnesium.

The preparation of the pure glycocholic acids may be performed in several ways. The bile, which has been freed from mucus by means of alcohol and the alcohol removed by evaporation, may be precipitated by a solution of lead acetate. The precipitate is then decomposed by a soda solution and heat, evaporated to dryness, and the residue extracted with alcohol, which dissolves the alkali glycocholate. The alcohol is distilled from the filtered solution and the residue dissolved in water; this solution is now decolorized by animal charcoal and the glycocholic acid precipitated from the solution by the addition of a dilute mineral acid. The mixture of the two glycocholic acids is freed from mineral acid by carefully washing with water, and then is boiled with water, when the glycocholic acid dissolves and may be obtained from the filtrate as crystals on cooling. The glycocholeic acid with some transformed glycocholic acid (paraglycocholic acid) remains undissolved and may be purified by converting it into the insoluble barium salt. If we do not care for the obtainment of pure glycocholeic acid but want only the pure glycocholic acid, then the decolorization with animal charcoal can be omitted. If the bile is rich in glycocholic acid, we can treat the mucus-free bile, according to HÜFNER'S[1] method, with ether and hydrochloric acid, when the glycocholic acid crystallizes out in abundance. The reader is referred to more exhaustive works for other methods of preparation.

Hyoglycocholic Acid, $C_{27}H_{43}NO_5$, is the crystalline glycocholic acid obtained from the bile of the pig. It is very insoluble in water. The alkali salts, whose solutions have an intensely bitter taste, without any sweetish after-taste, are precipitated by $CaCl_2$, $BaCl_2$, and $MgCl_2$, and may be salted out like a soap by Na_2SO_4 when added in sufficient quantity. By precipitation with NaCl in such quantity that the precipitate redissolves on warming, HAMMARSTEN[2] has obtained the alkali salt as macroscopic crystals on cooling. Besides this acid there occurs in the bile of the pig still another glycocholic acid (JOLIN[3]).

The glycocholate in the bile of the rodent is also precipitated by the above-mentioned earthy salts, but cannot, like the corresponding salt in human or ox bile, be directly precipitated on saturating with a neutral salt (Na_2SO_4). **Guano bile-acid** possibly belongs to the glycocholic-acid group, and is found in Peruvian guano, but has not been thoroughly studied.

Taurocholic Acid. This acid, which is found in the bile of man, carnivora, oxen, and a few other herbivora, such as sheep and goats, has the constitution $C_{26}H_{45}NSO_7$. On boiling with acids and alkalies it splits into cholic acid and taurine. Taurocholic acid has also been prepared synthetically by BONDI and MÜLLER, using the same method as they used for glycocholic acid.

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[1] Journ. f. prakt. Chem., 1874.

[2] Not published.

[3] Zeitschr. f. physiol. Chem., 12 and 13.

Taurocholic acid can be readily obtained, by the method suggested by HAMMARSTEN,[1] as groups of fine needles or as beautiful prisms on slow crystallization. The crystals do not change in the air, but they decompose above 100°. They are soluble in alcohol but insoluble in ether, benzene, and acetone. Taurocholic acid is very soluble in water, and the solution has a very sweet taste, with only a slight bitter taste. It can hold the difficultly soluble glycocholic acid in solution. This is the reason why a mixture of glycocholate with a sufficient quantity of taurocholate, which often occurs in ox-bile, is not precipitated by a dilute acid. Its salts are, as a rule, readily soluble in water, and the solutions of the alkali salts are not precipitated by copper sulphate, silver nitrate, or sugar of lead. Basic lead acetate gives, on the contrary, a precipitate which is soluble in boiling alcohol. The alkali salts are not only precipitated from their solution by the same neutral salts that precipitate glycocholic acid, but also by potassium chloride, and by sodium and potassium acetates.

Taurocholic acid is most simply prepared from a glycocholic-acid-free bile or from one very poor in this acid, such as fish- or dog-bile. From ox-bile it can be prepared by first precipitating the glycocholic acid with alum and then repeatedly precipitating the filtrate with ferric chloride (according to TENGSTRÖM). From this filtrate the taurocholate is precipitated by saturating with NaCl, the precipitate pressed and freed from NaCl by dissolving in alcohol, and as a powder, or dissolved in a little alcohol, is decomposed by alcohol containing hydrochloric acid. The acid is precipitated from the filtrate by ether. The taurocholic acid can be repeatedly recrystallized by solution in alcohol containing water and the careful addition of ether.

Taurocholeic Acid is a second taurocholic acid, detected by HAMMARSTEN in dog-bile and isolated by GULLBRING[2] from ox-bile, and has the formula $C_{26}H_{45}NSO_6$ or $C_{27}H_{47}NSO_6$. Thus far it has been obtained only in the amorphous form. It is readily soluble in water, and has a disagreeably bitter taste. It is also readily soluble in alcohol, but insoluble in ether, acetone, chloroform, and benzene. The alkali salt, soluble in water, can be salted out by NaCl as a pasty mass. The solutions of the salts can be precipitated by ferric chloride. The cleavage products are taurine and choleic acid.

For the preparation of taurocholeic acid it is best to use dog-bile which is first precipitated by ferric chloride. The precipitate contains the acid, while the filtrate can be used for the obtainment of taurocholic acid by saturating with NaCl. The iron precipitate is converted into the alkali salt by sodium carbonate, and is decomposed by alcohol containing hydrochloric acid, and then precipitated by ether. The amorphous acid which separates is purified from alcoholic solution by precipitation with ether. In preparing taurocholic acid from ox-bile, the taurocholeic acid, which is readily soluble in alcohol-ether, remains in the alcohol-ether on the proper addition of ether. This crude acid as alkali salt is freed from taurocholic acid by precipitation with ferric chloride, then again converted into alkali salt, decomposed with acid in alcohol, precipitated by ether, and purified (GULLBRING).

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[1] Zeitschr. f. physiol. Chem., **43**.

[2] Hammarsten, Zeitschr. f. physiol. Chem., **43**; Gullbring, *ibid.*, **45**.

Cheno-taurocholic Acid. This is the most essential acid of goose-bile and has the formula $C_{29}H_{49}NSO_6$. This acid, though little studied, is amorphous and soluble in water and alcohol.

The taurocholic acids differ from the glycocholic acids in being readily soluble in water. In the bile of the walrus, on the contrary, a relatively insoluble, readily crystallizable taurocholic acid occurs which can be precipitated from the solution of the alkali salts by the addition of mineral acids, similar to glycocholic acid (HAMMARSTEN[1]).

As repeatedly mentioned above, the two bile-acids split on boiling with acids or alkalies into non-nitrogenous cholic acids and glycocoll or taurine. Of the various cholic acids the following have been best studied.

Cholic Acid or Cholalic Acid. The ordinary cholic acid obtained as a decomposition product of human and ox bile, which occurs regularly in the contents of the intestine and in the urine in icterus, has, according to STRECKER and nearly all recent investigators, the constitution $C_{24}H_{40}O_5 =$ $C_{20}H_{31}\left\{\begin{array}{l}CHOH\\(CH_2OH)_2\\COOH\end{array}\right.$. According to MYLIUS,[2] cholic acid is a monobasic alcohol-acid with one secondary and two primary alcohol groups. CURTIUS[3] has shown by preparing the cholamine, $C_{23}H_{39}O_3NH_2$, from the above-mentioned (p. 312) cholic-acid azide, with cholic-acid urethane as an intermediary step, that the carboxyl group is not immediately connected with the CHOH group, but is combined with the chief nucleus without the neighboring secondary alcohol group. On oxidation it first yields *dehydrocholic acid*, $C_{24}H_{34}O_5$ (HAMMARSTEN). On further oxidation *bilianic acid*, $C_{24}H_{34}O_8$ (CLEVE), is obtained, or, more correctly, according to LASSAR-COHN and PREGL, a mixture of bilianic and *isobilianic* acids. On oxidation, bilianic acid yields *cilianic acid* (LASSAR-COHN), whose formula, according to PREGL, is $C_{20}H_{28}O_8$. On stronger oxidation it yields *cholesterinic acid*, which has not been carefully studied, and finally phthalic acid, as maintained by SÉNKOWSKI, but not substantiated by BULNHEIM or PREGL.[1] On reduction (in putrefaction) cholic acid may yield *desoxycholic acid*, $C_{24}H_{40}O_4$ (MYLIUS). On reduction with hydriodic acid and red phosphorus, PREGL obtained a product which he considers as a mono-carboxylic acid, with the formula $C_{20}H_{31}\left\{\begin{array}{l}CH_2\\(CH_3)_2\\COOH\end{array}\right.$. SÉNKOWSKI has obtained an acid with the formula $C_{24}H_{40}O_2$, *cholylic acid*, on the reduction of the anhydride.[2]

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[1] Not published.

[2] The important researches of Strecker on the bile-acids may be found in Annal. d. Chem. u. Pharm., 65, 67, and 70; Mylius, Ber. d. deutsch. chem. Gesellsch., 19.

[3] *Ibid.*, 39.

As above mentioned, PREGL[3] has obtained, by the action of concentrated sulphuric acid upon cholic acid, a fluorescent substance which he calls dehydrocholon. This is produced by oxidation, and at the same time, water is eliminated. It has probably the formula $C_{24}H_{38}O$. Dehydrocholon is nitrated by nitric acid, while the cholic acid is not. From this behavior, as well as from the determination of the molecular refraction and dispersion of both bodies, PREGL finds it probable that cholic acid belongs to the hydrated carbocyclic compounds.

Cholic acid crystallizes partly in rhombic plates or prisms with 1 molecule of water and partly in larger rhombic tetrahedra or octahedra with 1 molecule of alcohol of crystallization (MYLIUS). These crystals become quickly opaque and porcelain-white in the air. They are quite insoluble in water (in 4000 parts cold and 750 parts boiling), rather soluble in alcohol, but soluble with difficulty in ether. The amorphous cholic acid is less insoluble. The solutions have a bitter-sweetish taste. The crystals lose their alcohol of crystallization only after a lengthy heating to 100–120° C. The acid free from water and alcohol melts at 195° C. According to BONDI and MÜLLER the melting-point of the perfectly pure acid is 198°. It forms a characteristic blue compound with iodine (MYLIUS).

The alkali salts are readily soluble in water, but when treated with a concentrated caustic or carbonated alkali solution may be separated as an oily mass which becomes crystalline on cooling. The alkali salts are not readily soluble in alcohol, and on the evaporation of the alcohol they may crystallize. The specific rotatory power of the sodium salt is $(\alpha)_D = +31.4°$.[4] The watery solution of the alkali salts, when not too dilute, is precipitated immediately or after some time by sugar of lead or by barium chloride. The barium salt crystallizes in fine, silky needles, and it is rather insoluble in cold, but somewhat easily soluble in warm water. The barium salt, as well as the lead salt which is insoluble in water, is soluble in warm alcohol.

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[1] Hammarsten, Ber. d. deutsch. chem. Gesellsch., **14**; Cleve, Bull. Soc. chim., **35**; Lassar-Cohn, Ber. d. d. chem. Gesellsch., **32**; Pregl, Wien. Sitzungsber., **111**, 1902; Sénkowski, Monatshefte f. Chem., **17**; Bulnheim, Zeitschr. f. physiol. Chem., **25**, in which the literature on cholesterinic acid may be found.

[2] Mylius, l. c.; Pregl, Pflüger's Arch., **71**; Sénkowski, Monatshefte f. Chem., **19**.

[3] Zeitschr. f. physiol. Chem., **45**.

[4] See Vahlen, Zeitschr. f. physiol. Chem., **21**.

Choleic Acid ($C_{25}H_{42}O_4$, LATSCHINOFF) is another cholic acid which, according to LASSAR-COHN,[1] has the formula $C_{24}H_{40}O_4$. This acid, which occurs in varying but always small quantities in ox-bile, yields *dehydrocholeic acid*, $C_{24}H_{34}O_4$, and then *cholanic acid*, $C_{24}H_{34}O_7$, and *isocholanic acid* on oxidation.

Choleic acid crystallizes when free from water in hexagonal, vitreous prisms with pointed ends, melting at 185–190° C. The crystalline acid containing water melts at 135–140° C. (LATSCHINOFF). The acid dissolves in water with difficulty and is also relatively difficultly soluble in alcohol. It has an intensely bitter taste and gives the MYLIUS iodine reaction for cholic acid. The specific rotation is $(\alpha)_D = +48.87^\circ$ (VAHLEN). The barium salt which crystallizes from the hot alcoholic solution as spherical aggregations of radial needles is more difficultly soluble in water than the corresponding cholate.

The relation of choleic acid to **desoxycholic acid** is not known. According to LATSCHINOFF and LASSAR-COHN both acids are identical, while PREGL,[2] on the contrary, claims that desoxycholic acid is more readily soluble in water and when anhydrous has a melting-point of 172–173°. According to the ordinary views the desoxycholic acid is formed from cholic acid by reduction. EKBOM[3] could not substantiate this statement. On using perfectly pure cholic acid he was able to regain nearly quantitatively after the action of metallic sodium on the alcoholic solution of the acid or of zinc and alkali. By treatment with zinc and acetic acid a reaction took place, but the product was a mixture of mono- and diacetyl derivatives. This indicates that desoxycholic acid is isomeric with an acid preformed in the bile, either choleic acid or possibly, as PREGL has shown, an acid isomeric therewith. The observation of PREGL that desoxycholic acid, like choleic acid, yields dehydrocholeic acid and cholanic acid as oxidation products, stands in close connection with such an assumption, but makes the formation of desoxycholic acid from cholic acid by reduction very improbable.

Both cholic acids are best prepared from ox-bile which has been boiled for twenty-four hours with baryta-water or caustic soda. According to MYLIUS,[4] boil the bile for twenty-four hours with five times its weight of a 30 per cent caustic-soda solution, replacing the water lost by evaporation.

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[1] Latschinoff, Ber. d. deutsch. chem. Gesellsch., **18** and **20**; Lassar-Cohn, *ibid.*, **26**, and Zeitschr. f. physiol. Chem., **17**. See also Vahlen. Zeitschr. f. physiol. Chem., **23**.

[2] Wien. Sitzungsber., **111**. Math. Naturw. Kl. 1902; Latschinoff, l. c.; Lassar-Cohn, l. c. See also Mylius, Ber. d. d. chem. Gesellsch., **19**.

[3] Unpublished investigation.

[4] Zeitschr. f. physiol. Chem., **12**.

Now saturate the liquid with CO_2 and evaporate nearly to dryness. The residue is extracted with 96 per cent alcohol and this alcoholic extract diluted with water until it contains at the most 20 per cent alcohol; it is then completely precipitated with a $BaCl_2$ solution. The precipitate, which contains besides fatty acids also the choleic acid, is filtered, and the cholic acid, contaminated with choleic acid, is precipitated from the filtrate by hydrochloric acid. After the cholic acid has gradually crystallized out it is repeatedly recrystallized from alcohol or methyl alcohol. According to Bondi and Müller,[1] perfectly pure cholic acid having a melting-point of 198° can be obtained by boiling the impure acid for four hours with 10 per cent caustic soda, reprecipitating with hydrochloric acid, and recrystallizing from alcohol.

Choleic acid may be obtained from the above-mentioned barium precipitate by first converting the barium salt into sodium salt by sodium carbonate, then fractionally precipitating the fatty acids by barium acetate, separating the choleic acid from the filtrate by hydrochloric acid and recrystallizing several times from glacial acetic acid.

Pregl[2] has suggested a somewhat different but simpler method for preparing cholic acid and obtaining the desoxycholic acid from ox-bile. In regard to this as well as other methods of preparation we must refer to the original communications and to other handbooks.

Fellic Acid, $C_{23}H_{40}O_4$, is a cholic acid, so called by Schotten, which he obtained from human bile, along with the ordinary acid. This acid is crystalline, is insoluble in water, and yields barium and magnesium salts which are very insoluble. It does not respond to Pettenkofer's reaction easily and gives a more reddish-blue color.

The conjugate acids of human bile have not been sufficiently investigated. To all appearances human bile contains under different circumstances various conjugate bile-acids. In some cases the bile-salts of human bile are precipitated by $BaCl_2$ and in others not. According to the statements of Lassar-Cohn[3] three cholic acids may be prepared from human bile, namely, ordinary CHOLIC ACID, CHOLEIC ACID, and FELLIC ACID.

Lithofellic Acid, $C_{20}H_{36}O_4$, is the acid related to cholic acid which occurs in the oriental bezoar stones, which is insoluble in water, comparatively easily soluble in alcohol, but only slightly soluble in ether.[4]

The hyo-glycocholic and cheno-taurocholic acids, as well as the glycocholic acid of the bile of rodents, yield corresponding cholic acids. This seems to be the case also with the glycocholic acid of the hippopotamus-bile, which stands very close to the pig-bile (Hammarsten[5]). In the polar bear a third cholic acid exists besides cholic and choleic acids. It is called *ursocholeic acid*, $C_{19}H_{30}O_4$ or $C_{18}H_{28}O_4$ (HAMMARSTEN[1]). The bile of other animals (walrus, sea-dog) contains special cholic acids (HAMMARSTEN[2]).

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[1] Zeitschr. f. physiol. Chem., **47**.

[2] l. c., Wien. Sitzungsber.

[3] Schotten, Zeitschr. f. physiol. Chem., **11**; Lassar-Cohn, Ber. d. deutsch. chem. Gesellsch., **27**.

[4] See Jünger and Klages, Ber. d. deutsch. chem. Gesellsch., **28** (older literature).

[5] Investigations not published.

On boiling with acids, on putrefaction in the intestine, or on heating, cholic acids lose water and are converted into anhydrides, the so-called *dyslysins*. The dyslysin, $C_{24}H_{36}O_3$, corresponding to ordinary cholic acid, which occurs in fæces, is amorphous, insoluble in water and alkalies. *Choloidic acid*, $C_{24}H_{38}O_4$, is called the first anhydride or an intermediary product in the formation of dyslysin. On boiling dyslysins with caustic alkali they are reconverted into the corresponding cholic acids.

THE DETECTION OF BILE-ACIDS IN ANIMAL FLUIDS. To obtain the bile-acids pure so that PETTENKOFER'S test can be applied to them, the protein and fat must first be removed. The protein is removed by making the liquid first neutral and then adding a great excess of alcohol, so that the mixture contains at least 85 vols. per cent of water-free alcohol. Now filter, extract the precipitated protein with fresh alcohol, unite all filtrates, distil the alcohol, and evaporate to dryness. The residue is completely exhausted with strong alcohol, filtered, and the alcohol entirely evaporated from the filtrate. The new residue is dissolved in water, and filtered if necessary, and the solution precipitated by basic lead acetate and ammonia. The washed precipitate is dissolved in boiling alcohol, filtered while warm, and a few drops of soda solution added. Then evaporate to dryness, extract the residue with absolute alcohol, filter, and add an excess of ether. The precipitate now formed may be used for PETTENKOFER'S test. It is not necessary to wait for crystallization; but one must not consider the crystals which form in the liquid as being positively crystallized bile. It is also possible for needles of alkali acetate to be formed. For the detection of bile-acids in urine see Chapter XV.

Bile-pigments. The bile-coloring matters known thus far are relatively numerous, and in all probability there are still more of them. Most of the known bile-pigments are not found in the normal bile, but occur either in postmortem bile or principally in the bile concrements. The pigments which occur under physiological conditions are the reddish-yellow *bilirubin*, the green *biliverdin*, and sometimes also *urobilin* (and *urobilinogen*) or a closely related pigment. The pigments found in gall-stones are (besides the *bilirubin* and *biliverdin*) *choleprasin*, *bilifuscin*, *biliprasin*, *bilihumin*, *bilicyanin* (and *choletelin?*). Besides these, others have been noticed in human and animal bile by various observers. The two above-mentioned physiological pigments, bilirubin and biliverdin, are those which serve to give the golden-yellow or orange-yellow or sometimes greenish color to the bile; or when, as is most frequently the case in ox-bile, the two pigments are present in the bile at the same time, they produce the different shades between reddish brown and green.

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[1] Zeitschr. f. physiol. Chem., 36.

[2] Investigations not published.

Bilirubin. This pigment has the formula $C_{16}H_{18}N_2O_3$, or according to ORNDORFF and TEEPLE[1] more correctly $C_{32}H_{36}N_4O_6$, and is designated by the names CHOLEPYRRHIN, BILIPHÆIN, BILIFULVIN, and HÆMATOIDIN. It occurs chiefly in the gall-stones as calcium bilirubin. Bilirubin is present in the liver-bile of all vertebrates, and in the bladder-bile especially in man and carnivora; sometimes, however, the latter may have a green bile when fasting or in a starving condition. It occurs also in the contents of the small intestine, in the blood-serum of the horse, in old blood extravasations (as hæmatoidin), and in the urine and the yellow-colored tissue in icterus. It is converted into *hydrobilirubin*, $C_{32}H_{40}N_4O_7$ (MALY), by hydrogen in a nascent state, and then shows great similarity to the urinary pigment, *urobilin*, as well as to *stercobilin* found in the contents of the intestine (MASIUS and VANLAIR[2]). On careful oxidation bilirubin yields biliverdin and other coloring-matters (see below).

Bilirubin is derived from the blood-pigment. It has the same percentage composition as hæmatoporphyrin, and like hæmatin it yields hæmatinic-acid imide as an oxidation product (KÜSTER). On reduction with zinc powder or with nascent HI, it yields hæmopyrrol according to ORNDORFF and TEEPLE.[3]

Bilirubin is sometimes amorphous and sometimes crystalline. The amorphous bilirubin is a reddish-yellow or reddish-brown powder; the crystals have a reddish-yellow, reddish-brown, or more reddish color, and sometimes they have nearly the color of crystalline chromic acid. The crystals, which can easily be obtained by allowing a solution of bilirubin in chloroform to evaporate spontaneously, are reddish-yellow, rhombic plates, whose obtuse angles are often rounded. On crystallizing from hot dimethylaniline it forms on cooling broad columns with both ends sharply cut (KÜSTER[4]). On dissolving in chloroform both kinds of crystals are converted into long needles or whetstones.

Bilirubin is insoluble in water, behaves like an acid, and occurs in animal fluids as soluble alkali bilirubin. It is very slightly soluble in ether, benzene, carbon disulphide, amyl alcohol, fatty oils, and glycerine. It is somewhat more soluble in alcohol. In cold chloroform it dissolves with difficulty and is much more readily soluble in warm chloroform. Its solubility varies, and supersaturated solutions are readily formed (ORNDORFF and TEEPLE). The varying solubility of bilirubin in chloroform depends, according to Küster, on the fact that in its preparation derivatives which are readily soluble and contain chlorine or other transformation products are formed, or perhaps the bilirubin goes over into polymeric modifications having different solubilities. In cold dimethylaniline it dissolves in the proportion of 1:100, and in hot dimethylaniline much more readily. Its solutions show no absorption-bands, but only a continuous absorption from the red to the violet end of the spectrum, and they have, even on diluting greatly (1:500 000), in a layer 1.5 cm. thick a decided yellow color. If a dilute solution of alkali bilirubin in water is treated with an excess of ammonia and then with a zinc-chloride solution, the liquid is first colored deep orange and then gradually olive-brown and then green. This solution first gives a darkening of the violet and blue part of the spectrum and then the bands of alkaline cholecyanin (see below), or at least the bands of this pigment in the red between *C* and *D*, close to *C*. This is a good reaction for bilirubin. The compounds of bilirubin with alkalies are insoluble in chloroform, and bilirubin may be separated from its solution in chloroform by shaking with dilute caustic alkali (differing from lutein). Solutions of alkali bilirubin in water are precipitated by the soluble salts of the alkaline earths and also by metallic salts.

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[1] Salkowski's Festschrift, Berlin, 1904.

[2] Maly, Wien. Sitzungsber., 57, and Annal. d. Chem., **163**; Masius and Vanlair, Centralbl. f. d. med. Wissensch., 1871, 369.

[3] l. c.

[4] Ber. d. d. chem. Gesellsch., **30** and **35**, and Zeitschr. f. physiol. Chem., **47**.

As Ehrlich first showed, bilirubin forms combinations with diazo compounds, which have been closely studied by Pröscher, Orndorff and Teeple.[1] A test suggested by Ehrlich for bilirubin is based upon this behavior with diazobenzenesulphonic acid.

If an alkaline solution of bilirubin be allowed to stand in contact with the air, it gradually absorbs oxygen, and green biliverdin is formed. This process is accelerated by warming. According to Küster, in this case the alkali also has a splitting action upon the pigment, and not one body but several are formed. Biliverdin is also formed from bilirubin by oxidation under other conditions. A green coloring-matter similar in appearance is formed by the action of other reagents such as Cl, Br, and I. According to Jolles,[2] by the use of Hübl's iodine solution biliverdin is produced, while according to others (Thudichum, Maly [3]) substitution products of bilirubin are formed.

Gmelin's *Reaction for Bile-pigments.* If one carefully pours under an aqueous solution of alkali bilirubin nitric acid containing some nitrous acid, there is obtained a series of colored layers at the juncture of the two liquids in the following order from above downwards: green, blue, violet, red, and reddish yellow. This color reaction, GMELIN's test, is very delicate and serves to detect the presence of one part bilirubin in 80 000 parts liquid. The green ring must never be absent; and also the reddish-violet must be present at the same time, otherwise the reaction may be confused with that for lutein, which gives a blue or greenish ring. The nitric acid must not contain too much nitrous acid, for then the reaction takes place too quickly and it does not become typical. Alcohol must not be present in the liquid, because, as is well known, it gives a play of colors, in green or blue, with the acid.

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[1] Ehrlich, Zeitschr. f. anal. Chem., **23**; Pröscher, Zeitschr. f. physiol. Chem., **39**; Orndorff and Teeple, l. c.

[2] Küster, Ber. d. d. chem. Gesellsch., **35**; Jolles, Journ. f. prakt. Chem. (N. F.), **59**, and Pflüger's Arch., **75**.

[3] Thudichum, Journ. of Chem. Soc. (2), **13**, and Journ. f. prakt. Chem. (N. F.), **53**; Maly, Wien. Sitzungsber., **72**.

HAMMARSTEN's *Reaction.* An acid is first prepared consisting of 1 vol. nitric acid and 19 vols. hydrochloric acid (each acid being about 25 per cent). One volume of this acid mixture, which can be kept for at least a year, is, when it has become yellow by standing, mixed with 4 vols. alcohol. If a drop of bilirubin solution is added to a few cubic centimetres of this colorless mixture a permanent beautiful green color is obtained immediately. On the further addition of the acid mixture to the green liquid all the colors of GMELIN's scale, as far as choletelin, can be produced consecutively.

HUPPERT's *Reaction.* If a solution of alkali bilirubin is treated with milk of lime or with calcium chloride and ammonia, a precipitate is produced consisting of calcium bilirubin. If this moist precipitate, which has been washed with water, is placed in a test-tube and the tube half filled with alcohol which has been acidified with hydrochloric acid, and heated to boiling for some time, the liquid becomes emerald-green or bluish green in color.

In regard to the modifications of GMELIN's test and certain other reactions for bile-pigments, see Chapter XV (Urine).

That the characteristic play of colors in GMELIN's test is the result of an oxidation is generally admitted. The first oxidation step is the green biliverdin. Then follows a blue coloring-matter which HEINSIUS and CAMPBELL call *bilicyanin* and STOKVIS calls *cholecyanin*, and which shows a characteristic absorption-spectrum. The neutral solutions of this coloring-matter are, according to STOKVIS, bluish green or steel-blue with a beautiful blue fluorescence. The alkaline solutions are green and have no marked fluorescence, and show three absorption-bands: one, sharp and dark, in the red between *C* and *D*, nearer to *C*; a second, less well defined, covering *D*; and a third between *E* and *F*, near *E*. The strongly acid solutions are violet-blue and show two bands, described by JAFFÉ, between the lines *C* and *E*, separated from each other by a narrow space near *D*. A third band between *b* and *F* is seen with difficulty. The next oxidation step after these blue coloring-matters is a red pigment, and lastly a yellowish-brown pigment, called *choletelin* by MALY, which in neutral alcoholic solutions does not give any absorption-spectrum, but in acid solution gives a band between *b* and *F*. On oxidizing cholecyanin with lead peroxide, STOKVIS[1] obtained a product which he calls choletelin, which is quite similar to urinary urobilin, to be discussed later.

Bilirubin is best prepared from gall-stones of oxen, these concretions being very rich in calcium bilirubin. The finely powdered concrement is first exhausted with ether and then with boiling water, so as to remove the cholesterin and bile-acids. In order to remove the mineral constituents it is better to use 10 per cent acetic acid instead of hydrochloric acid (KÜSTER[2]). A green pigment is now removed by extraction with alcohol, and the choleprasin is extracted with hot glacial acetic acid. After washing with water it is dried, and extracted repeatedly with boiling chloroform. The bilirubin separates from the chloroform as crusts, which are treated once or twice in the above manner. It is then extracted with alcohol and precipitated from its chloroform solution by alcohol or crystallized from dimethylaniline.

The chloroform solution which separates from the crusts of bilirubin contains, according to KÜSTER, a pigment related to bilirubin, poorer in nitrogen, also precipitable by alcohol, and very readily soluble in chloroform. This has been substantiated by ORNDORFF and TEEPLE.[3] This pigment, according to KÜSTER, is a transformation product of bilirubin which is rich in chlorine.

The quantitative estimation of bilirubin may be made by the spectrophotometric method, according to the steps suggested for the blood-coloring matters.

Biliverdin, $C_{16}H_{18}N_2O_4$ or $C_{32}H_{36}N_4O_8$. This body, which is formed by the oxidation of bilirubin, occurs in the bile of many animals, in vomited matter, in the placenta of the bitch (?), in the shells of birds' eggs, in the urine in icterus, and sometimes in gall-stones, although in very small quantities.

Biliverdin is amorphous; at least it has not been obtained in well-defined crystals. It is insoluble in water, ether, and chloroform (this is true at least for the artificially prepared biliverdin), but is soluble in alcohol or glacial acetic acid, showing a beautiful green color. It is dissolved by alkalies, giving a brownish-green color, and this solution is precipitated by acids, as well as by calcium, barium, and lead salts. Biliverdin gives HUPPERT'S, GMELIN'S, and HAMMARSTEN'S reactions, commencing with the blue color. It is converted into hydrobilirubin by nascent hydrogen. On allowing the green bile to stand, also by the action of ammonium sulphide, the biliverdin may be reduced to bilirubin (HAYCRAFT and SCOFIELD[4]).

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[1] Heinsius and Campbell, Pflüger's Arch., 4; Stokvis, Centralbl. f. d. med. Wissensch., 1872, 785; *ibid.*, 1873, 211 and 449; Jaffé, *ibid.*, 1868; Maly, Wien. Sitzungsber., 59.

[2] Zeitschr. f. physiol. Chem., 47.

[3] Küster, Ber. d. d. chem. Gesellsch., 35; Orndorff and Teeple, l. c.

[4] Centralbl. f Physiol., 3, 222, and Zeitschr. f. physiol. Chem., 14.

Biliverdin is most simply prepared by allowing a thin layer of an alkaline solution of bilirubin to stand exposed to the air in a dish until the color is brownish green. The solution is then precipitated by hydrochloric acid, the precipitate washed with water until no HCl reaction is obtained, then dissolved in alcohol and the pigment again separated by the addition of water. Any bilirubin present may be removed by means of chloroform. Hugounenq and Doyon[1] prepared biliverdin from bilirubin by the action of sodium peroxide and a little acid.

Choleprasin is a green pigment isolated by Küster[2] from gall-stones, which is soluble in glacial acetic acid but insoluble in alcohol. It differs from the other bile-pigments by containing sulphur. On distillation with zinc powder it gives the pyrrol reaction, and on oxidation with chromic acid, Küster could not observe any formation of hæmatinic acid.

Bilifuscin, so named by Städeler,[3] is an amorphous brown pigment soluble in alcohol and alkalies, nearly insoluble in water and ether, and soluble with great difficulty in chloroform (when bilirubin is not present at the same time). Pure bilifuscin does not give Gmelin's reaction. This is also true for the bilifuscin prepared by v. Zumbusch,[4] which is more like a humin substance and the formula of which is $C_{64}H_{96}N_7O_{14}$. Bilifuscin has been found in gall-stones. *Biliprasin* is a green pigment prepared by Städeler from gall-stones, which is generally considered as a mixture of biliverdin and bilirubin. Dastre and Floresco,[5] on the contrary, consider biliprasin as an intermediate step between bilirubin and biliverdin. According to them it occurs as a physiological pigment in the bladder-bile of several animals and is derived from bilirubin by oxidation. This oxidation is brought about by an oxidative ferment existing in the bile. *Bilihumin* is the name given by Städeler to that brownish amorphous residue which is left after extracting gall-stones with chloroform, alcohol, and ether. It does not give Gmelin's test. *Bilicyanin* is also found in human gall-stones (Heinsius and Campbell). *Cholohæmatin*, so called by MacMunn, is a pigment often occurring in sheep- and ox-bile and characterized by four absorption-bands, which is formed from hæmatin by the action of sodium amalgam. In the dried condition, as when obtained by the evaporation of the chloroform solution, it is green, and in alcoholic solution olive-brown. This pigment, which has also been found by Hammarsten in the bile from the musk-ox and hippopotamus, is, according to Marchlewski, identical with the crystalline *bilipurpurin* isolated by Loebisch and Fischler from ox-bile. This latter pigment, according to Marchlewski, is not a bile-pigment, but *phylloerythrin*, a transformation product of chlorophyll. Phylloerythrin has been detected by Marchlewski[6] in the excrement of cows fed on green grass.

Gmelin's and Huppert's reactions are generally used to detect the presence of bile-pigments in animal fluids or tissues. The first, as a rule, can be performed directly, and the presence of proteins does not interfere with it, but, on the contrary, it brings out the play of colors more strikingly. If blood-coloring matters are present at the same time, the bile-coloring matters are first precipitated by the addition of sodium phosphate and milk of lime. This precipitate containing the bile-pigments may be used directly in HUPPERT's reaction, or a little of the precipitate may be dissolved in HAMMARSTEN's reagent. Bilirubin is detected in blood, according to HEDENIUS,[1] by precipitating the proteins with alcohol, filtering and acidifying the filtrate with hydrochloric or sulphuric acid, and boiling. The liquid becomes of a greenish color. Serum and serous fluids may be boiled directly with a little acid after the addition of alcohol.

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[1] Arch. de Physiol. (5), 8.

[2] Zeitschr. f. physiol. Chem., **47**.

[3] Cited from Hoppe-Seyler, Physiol. u. Path. chem. Analyse, 6. Aufl., p. 225.

[4] Zeitschr. f. physiol. Chem., **31**.

[5] Arch. de Physiol. (5), **9**.

[6] MacMunn, Journ. of Physiol., **6**; Loebisch and Fischler, Wien. Sitzungsber., **112** (1903); Marchlewski, Zeitschr. f. physiol. Chem., **41**, **43**, and **45**; Hammarsten, *ibid.*, **43**, and investigations not published.

Besides the bile-acids and the bile-pigments, there occur in the bile also *cholesterin, lecithin, jecorin* or other *phosphatides* (HAMMARSTEN), *palmitin, stearin, olein, myristic acid* (LASSAR-COHN [2]), *soaps, ethereal sulphuric acids, conjugated glucuronates, diastatic* and *proteolytic enzymes. Choline* and *glycerophosphoric acid,* when they are present, may be considered as decomposition products of lecithin. *Urea* occurs, though only in traces, as a physiological constituent of human, ox, and dog bile. Urea occurs in the bile of the shark and ray in such large quantities that it forms one of the chief constituents of the bile.[3] The *mineral constituents* of the bile are, besides the alkalies, to which the bile-acids are united, sodium and potassium chloride, calcium and magnesium phosphate, and iron—0.04–0.115 p. m. in human bile, chiefly combined with phosphoric acid (YOUNG [4]). Traces of copper are habitually present, and traces of zinc are often found. Sulphates are entirely absent, or occur only in very small amounts.

The quantity of iron in the bile varies greatly. According to NOVI it is dependent upon the kind of food, and in dogs it is lowest with a bread diet and highest with a meat diet. According to DASTRE this is not the case. The quantity of iron in the bile varies even though a constant diet is maintained, and the variation is dependent upon the formation and destruction of blood. According to BECCARI [5] the iron does not disappear from the bile in inanition, and the percentage shows no constant diminution. The question as to the extent of elimination by the bile of the iron introduced into the body has received various answers. There is no doubt that the liver has the property of collecting and retaining iron as well as other metals from the blood. Certain investigators, such as NOVI and KUNKEL, are of the opinion that the iron introduced and transitorily retained in the liver is eliminated by the bile, while others, such as HAM-

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[1] Upsala Läkaref. Förh., **29**, and Maly's Jahresber., **24**.

[2] Zeitschr. f. physiol. Chem., **17**; Hammersten, *ibid.*, **32**, **36** and **43**.

[3] Hammarsten, *ibid.*, **24**.

[4] Journ. of Anat. and Physiol., **5**, 158.

[5] Novi, see Maly's Jahresber., **20**; Dastre, Arch. de Physiol. (5), **3**; Beccari, Arch. ital. de Biol., **28**.

BURGER, GOTTLIEB, and ANSELM,[1] deny any such elimination of iron by the bile.

Quantitative Composition of the Bile. Complete analyses of human bile have been made by HOPPE-SEYLER and his pupils. The bile was removed as fresh as possible from the gall-bladder of those cadavers the livers of which were in no sense pathological.

Older and less complete analyses of human bile have been made by FRERICHS and v. GORUP-BESANEZ.[2] The bile analyzed by them was from perfectly healthy persons who had been executed or accidentally killed. The two analyses of FRERICHS are, respectively, of (I) an 18-year-old and (II) a 22-year-old male. The analyses of v. GORUP-BESANEZ are of (I) a man of 49 and (II) a woman of 29. The results are, as usual, in parts per 1000.

	FRERICHS.		v. GORUP-BESANEZ.	
	I.	II.	I.	II.
Water	860.0	859.2	822.7	898.1
Solids	140.0	140.8	177.3	101.9
Biliary salts	72.2	91.4	107.9	56.5
Mucus and pigments	26.6	29.8	22.1	14.5
Cholesterin	1.6	2,6	47.3	30.9
Fat	3.2	9.2		
Inorganic substances	6.5	7.7	10.8	6.2

Human liver-bile is poorer in solids than the bladder-bile. In several cases it contained only 12–18 p. m. solids, but the bile in these cases is hardly to be considered as normal. JACOBSEN found 22.4–22.8 p. m. solids in a specimen of bile. HAMMARSTEN, who had occasion to analyze the liver-bile in seven cases of biliary fistula, has repeatedly found 25–28 p. m. solids. In a case of a corpulent woman the quantity of solids in the liver-bile varied between 30.10–38.6 p. m. in ten days. BRAND[3] has observed still higher figures, more than 40 p. m. in a couple of cases. This investigator suggests that the bile from an imperfect fistula, when it is partly absorbed, is richer in solids than when it comes from a perfect fistula.

The molecular concentration of human bile, according to BRAND, BONANNI, and STRAUSS,[4] is nearly always identical with that of the blood, although the amount of water and solids varies. The freezing-point varies only between −0.54° and −0.58°. This constancy of the osmotic pressure is explained by the fact that in concentrated biles with larger amounts of organic substances (with larger molecules) the amount of inorganic salts is lower.[1]

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[1] Kunkel, Pflüger's Arch., **14**; Hamburger, Zeitschr. f. physiol. Chem., **2** and **4**; Gottlieb, *ibid.*, **15**; Anselm, "Ueber die Eisenausscheidung der Galle," Inaug.-Diss. Dorpat, 1891. See also the works cited in foot-note 3, p. 244.

[2] See Hoppe-Seyler, Physiol. Chem., 301; Socoloff, Pflüger's Arch., **12**; Trifanowski, *ibid.*, **9**; Frerichs in Hoppe-Seyler's Physiol. Chem., 299; v. Gorup-Besanez, *ibid.*

[3] Jacobsen, Ber. d. deutsch. chem. Gesellsch., **6**; Hammarsten, Nova Acta Reg. Soc Scient. Upsala, **16**; Brand, Pflüger's Arch., **90**.

[4] Brand, l. c.; Bonanni, Biochem. Centralbl., **1**; Strauss, Berl. klin. Wochenschr., 1903.

Human bile sometimes, but not always, contains sulphur in an ethereal sulphuric-acid-like combination (Hammarsten, Oerum, Brand). The quantity of such sulphur may even amount to ¼–⅓ of the total sulphur. We do not know the nature of these ethereal sulphuric acids. According to Oerum[2] they are not precipitated by lead acetate, but are precipitated by basic lead acetate, especially with ammonia. Human bile is habitually richer in glycocholic than in taurocholic acid. In six cases of liver-bile analyzed by Hammarsten the relationship of taurocholic to glycocholic acid varied between 1:2.07 and 1:14.36. The bile analyzed by Jacobsen contained no taurocholic acid.

As an example of the composition of human liver-bile the following results of three analyses made by Hammarsten are given. The results are calculated in parts per 1000.[3]

Solids	25.200	35.260	25.400
Water	974.800	964.740	974.600
Mucin and pigments	5.290	4.290	5.150
Bile-salts	9.310	18.240	9.040
Taurocholate	3.034	2.079	2.180
Glycocholate	6.276	16.161	6.860
Fatty acids from soaps	1.230	1.360	1.010
Cholesterin	0.630	1.600	1.500
Lecithin	0.220	0.574	0.650
Fat		0.956	0.610
Soluble salts	8.070	6.760	7.250
Insoluble salts	0.250	0.490	0.210

Among the mineral constituents the chlorine and sodium occur to the greatest extent. The relationship between potassium and sodium varies considerably in different samples. Sulphuric acid and phosphoric acid occur only in very small quantities.

Baginsky and Sommerfeld[4] have found true mucin, mixed with some nucleoalbumin, in the bladder-bile of children. The bile contained on an average 896.5 p. m. water; 103.5 p. m. solids; 20 p. m. mucin; 9.1 p. m. mineral substances; 25.2 p. m. bile-salts (of which 16.3 p. m. were glycocholate and 8.9 p. m. taurocholate); 3.4 p. m. cholesterin; 6.7 p. m. fat, and 2.8 p. m. leucine.[5]

The quantity of pigment in human bile is, according to Noël-Paton, 0.4–1.3 p. m. (in a case of biliary fistula). The method used in determining the pigments in this case was not quite trustworthy. More exact results obtained by spectrophotometric methods are on record for dog-bile. According to STADELMANN [1] dog-bile contains on an average 0.6–0.7 p. m. bilirubin. At the most only 7 milligrams of pigment are secreted per kilo of body in the twenty-four hours.

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[1] See Brand, l. c.; Hammarsten, l. c.

[2] Skand. Archiv f. Physiol., 16.

[3] Recent quantitative analyses may be found in Brand, l. c.; v. Zeynek, Wien. klin. Wochenschr., 1899; Bonanni, l. c.

[4] Verhandl. d. physiol. Gesellsch. zu Berlin, 1894–95.

[5] Analyses of bile from children may be found in Heptner, Maly's Jahresber., 30.

In animals the relative proportion of the two acids varies considerably. It has been found, on determining the amount of sulphur, that, so far as the experiments have gone, taurocholic acid is the prevailing acid in carnivorous mammals, birds, snakes, and fishes. Among the herbivora, sheep and goats have a predominance of taurocholic acid in the bile. Ox-bile sometimes contains taurocholic acid in excess, in other cases glycocholic acid predominates, and in a few cases the latter occurs almost alone. The bile of the rabbit, hare, kangaroo, hippopotamus, and orang-utang (HAMMARSTEN [2]) contains, like the bile of the pig, almost exclusively glycocholic acid. A distinct influence on the relative amounts of the two bile-acids exerted by differences in diet has not been detected. RITTER [3] claims to have found a decrease in the quantity of taurocholic acid in calves when they pass from the milk to the vegetable diet.

In the above-mentioned calculation of the taurocholic acid from the quantity of sulphur in the bile-salts it must be remarked that no exact conclusion can be drawn from such a determination, since it is known that other kinds of bile (e.g., human and shark bile) contain sulphur in compounds other than taurocholic acid.[4]

The phosphorized constituents of bile are not well known; nevertheless, there is no doubt that bile contains other phosphatides besides lecithin (HAMMARSTEN). These phosphatides are in part precipitated in the precipitation of the bile-salts and they in part keep the bile-salts in solution, preventing their complete precipitation, and hence they have a double disturbing action in the quantitative analysis of bile. Those biles richest in phosphatides, so far as known, are the following, in the order of their amount: polar bear, man (in special cases), dog, black bear, orang-utang. The bile of certain fishes contains but little phosphatides (HAMMARSTEN [5]).

The cholesterin, which, according to several investigators, not only originates from the liver, but also from the biliary passages, occurs in larger quantities in the bladder-bile than in the liver-bile, and is present to a greater extent in the non-filtered than in the filtered bile (DOYON and DUFOURT[1]).

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[1] Noël-Paton, Rep. Lab. Roy. Soc. Coll. Phys. Edinburgh, **3**; Stadelmann, Der Icterus.

[2] Investigations not published. See Ergebnisse der Physiol., **4**.

[3] Cited from Maly's Jahresber., **6**, 195.

[4] Hammarsten, Zeitschr. f. physiol. Chem., **32**, and Ergebnisse der Physiol., **4**.

[5] Zeitschr. f. physiol. Chem., **36**, and Ergebnisse der Physiol., **4**.

The *gases* of the bile consist of a large quantity of carbon dioxide, which increases with the amount of alkalies, only traces of oxygen, and a very small quantity of nitrogen.

Little is known in regard to the *properties of the bile in disease*. The quantity of *urea* is found to be considerably increased in uræmia. *Leucine* and *tyrosine* are observed in acute yellow atrophy of the liver and in typhoid. Traces of *albumin* (without regard to nucleoalbumin) have several times been found in the human bile. The so-called *pigmentary acholia*, or the secretion of a bile containing bile-acids but no bile-pigments, has also been repeatedly noticed. In all such cases observed by RITTER he found a fatty degeneration of the liver-cells, in return for which, even in excessive fatty infiltration, a normal bile containing pigments was secreted. The secretion of a bile nearly free from bile-acids has been observed by HOPPE-SEYLER[2] in amyloid degeneration of the liver. In animals, dogs, and especially rabbits it has been observed that the blood-pigments pass into the bile in poisoning and in other conditions, causing a destruction of the blood-corpuscles, as also after intravenous hæmoglobin injection (WERTHEIMER and MEYER, FILEHNE, STERN[3]). Albumin can pass into the bile after the intravenous injection of a foreign protein (casein) (GÜRBER and HALLAUER), as well as after poisoning with phosphorus or arsenic (PILZECKER), or after the irritation of the liver by the introduction of ethyl alcohol or amyl alcohol (BRAUER). Sugar occurs in bile only in exceptional cases.[4]

The physiological secretion of the gall-bladder is according to WAHLGREN[5] in man a viscous, alkaline fluid with 11.24–19.63 p. m. solids The mucilaginous properties are not due to mucin but to a phosphorized protein substance (nucleoalbumin or nucleoproteid).

Instead of bile there is sometimes found in the gall-bladder under pathological conditions a more or less viscous, thready, colorless fluid which contains pseudomucins or other peculiar protein substances.[6]

Chemical Formation of the Bile. The first question to be answered is the following: Do the specific constituents of the bile, the bile-acids and bile-pigments, originate in the liver; and if this is the case, do they come from this organ alone, or are they also formed elsewhere?

The investigations of the blood, and especially the comparative investigations of the blood of the portal and hepatic veins under normal conditions, have not given any answer to this question. To decide this, therefore, it is necessary to extirpate the liver of animals or to isolate it from the circulation. If the bile constituents are not formed in the liver, or at least not alone in this organ, but are eliminated only from the blood, then, after the extirpation or removal of the liver from the circulation, an accumulation of the bile constituents is to be expected in the blood and tissues. If the bile constituents, on the contrary, are formed exclusively in the liver, then the above operation naturally would give no such result. If the ductus choledochus is tied, then the bile constituents will be collected in the blood or tissues whether they are formed in the liver or elsewhere.

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[1] Arch. de Physiol. (5), 8.

[2] Ritter, Compt. rend., 74, and Journ. de l'anat. et de la physiol. (Robin), 1872; Hoppe-Seyler, Physiol. Chem., 317.

[3] Wertheimer and Meyer, Compt. rend., **108**; Filehne, Virchow's Arch., **121**; Stern, *ibid.*, **123**.

[4] Gürber and Hallauer, Zeitschr. f. Biologie, 45; Pilzecker, Zeitschr. f. physiol. Chem., **41**; Brauer, *ibid.*, **40**.

[5] See Maly's Jahresber., 32.

[6] Winternitz, Zeitschr. f. physiol. Chem., 21; Sollmann, Amer. Medicine, 5 (1903)

From these principles **Köbner** has tried to demonstrate by experiments on frogs that the *bile-acids* are produced exclusively in the liver. While he was unable to detect any bile-acids in the blood and tissues of these animals after extirpation of the liver, he was able to discover them on tying the ductus choledochus. The investigations of **Ludwig** and **Fleischl**[1] show that in the dog the bile-acids originate in the liver alone. After tying the ductus choledochus they observed that the bile constituents were absorbed by the lymphatic vessels of the liver and passed into the blood through the thoracic duct. Bile-acids could be detected in the blood after such an operation, while they could not be detected in the normal blood. But when the common bile and thoracic ducts were both tied at the same time, then not the least trace of bile-acids could be detected in the blood, while if they are also formed in other organs and tissues they should have been present.

From older statements of **Cloez** and **Vulpian**, as well as **Virchow**, the bile-acids also occur in the suprarenal capsule. These statements have not been confirmed by later investigations of **Stadelmann** and **Beier**.[2] At the present time there is no ground for supposing that the bile-acids are formed elsewhere than in the liver.

It has been indubitably proved that the *bile-pigments* may be formed in other organs besides the liver, for, as is generally admitted, the coloring-matter hæmatoidin, which occurs in old blood extravasations, is identical with the bile-pigment bilirubin (see page 320). **Latschenberger**[3] has also observed in horses, under pathological conditions, a formation of bile-pigments from the blood-coloring matters in the tissues. Also the occurrence of bile-pigments in the placenta seems to depend on their formation in that organ, while the occurrence of small quantities of bile-pigments in the blood-serum of certain animals probably depends on an absorption of these substances.

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[1] Köbner, see Heidenhain, Physiologie der Absonderungsvorgänge, in Hermann's Handbuch, **5**; Fleischl, Arbeiten aus der physiol. Anstalt zu Leipzig, Jahrgang **9**.

[2] Zeitschr. f. physiol. Chem., **18**, in which the older literature may be found.

[3] See Maly's Jahresber., **16**, and Monatshefte f. Chem., **9**.

Although the bile-pigments may be formed in other organs besides the liver, still it is of first importance to know what bearing this organ has on the elimination and formation of bile-pigments. In this regard it must be recalled that the liver is an excretory organ for the bile-pigments circulating in the blood. TARCHANOFF has observed, in a dog with biliary fistula, that intravenous injection of bilirubin causes a very considerable increase in the bile-pigments eliminated. This statement has been confirmed lately by the investigations of VOSSIUS.[1]

Numerous experiments have been made to decide the question whether the bile-pigments are only eliminated by the liver or whether they are also formed therein. By experimenting on pigeons, STERN was able to detect bile-pigments in the blood-serum five hours after tying the biliary passages alone, while after tying all the vessels of the liver and also the biliary passages, no bile-pigments could be detected either in the blood or the tissues of the animal, which was killed 10–24 hours after the operation. MINKOWSKI and NAUNYN[2] have also found that poisoning with arseniuretted hydrogen produces a liberal formation of bile-pigments and the secretion, after a short time, of a urine rich in biliverdin in previously healthy geese. In geese with extirpated livers this does not occur.

No such experiments can be carried out on mammalia, as they do not live long enough after the operation; still there is no doubt that this organ is the chief seat of the formation of bile-pigments under physiological conditions.

In regard to the materials from which the bile-acids are produced, it may be said with certainty that the two components, glycocoll and taurine, which are both nitrogenized, are formed from the protein bodies. The close relationship of taurine to the cystine group of the protein molecule has been especially shown by the investigations of FRIEDMANN (see Chapter II), and very recently v. BERGMANN[3] has shown by feeding dogs with sodium cholate and cystine that the animal body can transform cystine into taurine and that the taurine of the bile originates from the proteins of the food. In regard to the origin of the non-nitrogenized cholic acid, which was formerly considered as originating from the fats, nothing is known positively.

The blood-coloring matters are considered as the mother-substances of the bile-pigments. If the identity of hæmatoidin and bilirubin was settled beyond a doubt, then this view might be considered as proved. Independently, however, of this identity, which is not admitted by all investigators, the view that the bile-pigments are derived from the blood-coloring matters has strong arguments in its favor. It has been shown by several experimenters that a yellow or yellowish-red pigment can be formed from the blood-coloring matters, which gives GMELIN's test, and which, though it may not form a complete bile-pigment, is at least a step in its formation (LATSCHENBERGER). A further proof of the formation of the bile-pigments from the blood-coloring matters consists in the fact that hæmatin on reduction yields urobilin, which is identical with hydrobilirubin (see Chapter XV). Further, hæmatoporphyrin (see page 212) and bilirubin are isomers, according to NENCKI and SIEBER, and closely allied. The formation of bilirubin from the blood-coloring matters is shown, according to the observations of several investigators,[1] by the appearance of free hæmoglobin in the plasma—produced by the destruction of the red corpuscles by widely differing influences (see below) or by the injection of hæmoglobin solution, causing an increased formation of bile-pigments. The amount of pigments in the bile is not only considerably increased, but the bile-pigments may even pass into the urine under certain circumstances (icterus). After the injection of hæmoglobin solution into a dog either subcutaneously or in the peritoneal cavity, STADELMANN and GORODECKI[2] observed in the secretion of pigments by the bile an increase of 61 per cent, which lasted for more than twenty-four hours.

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[1] Tarchanoff, Pflüger's Arch., 9; Vossius, cited from Stadelmann, Der Icterus.

[2] Stern, Arch. f. exp. Path. u. Pharm., 19; Minkowski and Naunyn, *ibid.*, 21.

[3] Hofmeister's Beiträge, 4. See also Wohlgemuth, Zeitschr. f. physiol. Chem., 40.

If bilirubin, which contains no iron, is derived from hæmatin, which contains iron, then iron must be split off. This process may be represented by the following formula, $C_{32}H_{34}N_4O_5Fe+H_2O-Fe=C_{32}H_{36}N_4O_6$. The question in what form or combination the iron is split off is of special interest, and also whether it is eliminated by the bile. This latter does not seem to be the case, at least to any great extent. In 100 parts of bilirubin which are eliminated by the bile there are only 1.4–1.5 parts iron, according to KUNKEL; while 100 parts hæmatin contain about 9 parts iron. MINKOWSKI and BASERIN[3] have also found that the abundant formation of bile-pigments occurring in poisoning by arseniuretted hydrogen does not increase the quantity of iron in the bile. The quantity apparently does not seem to correspond with that in the decomposed blood-coloring matters. It follows from the researches of several investigators[4] that the iron is, at least chiefly, retained by the liver as a ferruginous pigment or protein substance.

What relationship does the formation of bile-acids bear to the formation of bile-pigments? Are these two chief constituents of the bile derived simultaneously from the same material, and can we detect a certain connection between the formation of bilirubin and bile-acids in the liver? The investigations of STADELMANN teach us that this is not the case. With increased formation of bile-pigments the amount of bile-acids is decreased, and the introduction of hæmoglobin into the liver strongly increases the formation of bilirubin, but simultaneously strongly decreases the production of bile-acids. According to STADELMANN the formation of bile-pigments and bile-acids is due to a special activity of the cells.

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[1] See Stadelmann, Der Icterus, etc., Stuttgart, 1891.

[2] See Stadelmann, *ibid.*

[3] Kunkel, Pfluger's Arch., **14**; Minkowski and Baserin, Arch. f. exp. Path. u. Pharm., **23**.

[4] See Naunyn and Minkowski, Arch. f. exp. Path. u. Pharm., **21**; Latschenberger, l. c.; Neumann, Virchow's Arch., **111**, and the literature in foot-note 2, p. 282.

An absorption of bile from the liver and the passage of the bile constituents into the blood and urine occurs in retarded discharge of the bile, and usually in different forms of *hepatogenic icterus*. But bile-pigments may also pass into the urine under other circumstances, especially when in animals a solution or destruction of the red blood-corpuscles takes place through injection of water or a solution of biliary salts, through poisoning by ether, chloroform, arseniuretted hydrogen, phosphorus, or toluylenediamine, and in other cases. This occurs also in man in severe infectious diseases. It has also been claimed many times that a transformation of blood-pigments into bile-pigments occurs elsewhere than in the liver, namely, in the blood. Such a belief has been made very improbable by the important researches of MINKOWSKI and NAUNYN, AFANASSIEW, SILBERMANN, and especially of STADELMANN,[1] and in some of the above-mentioned cases, as after poisoning with phosphorus, toluylenediamine, and arseniuretted hydrogen, it has been disproved by direct experiment.

The icterus is also in these cases hepatogenic; it depends upon an absorption of bile-pigments from the liver, and this absorption seems to originate in various cases in somewhat different ways. Thus the bile may be viscous and cause a congestion of bile by counteracting the low secretion pressure. In other cases the fine biliary passages may be compressed by an abnormal swelling of the liver-cells, or a catarrh of the bile-passages may occur, causing a congestion of the bile (STADELMANN).

Bile Concretions.

The concrements which occur in the gall-bladder vary considerably in size, form, and number, and are of three kinds, depending upon the kind and nature of the bodies forming their chief mass. One group of gall-stones contains lime-pigment as chief constituent, another cholesterin, and the third calcium carbonate and phosphate. The concrements of the last-mentioned group occur very seldom in man. The so-called cholesterin-stones are those which occur most frequently in man, while the lime-pigment stones are not found very often in man, but often in oxen.

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[1] The literature belonging to this subject is found in Stadelmann, Der Icterus, etc., Stuttgart, 1891.

The *pigment-stones* are generally not large in man, but in oxen and pigs they are sometimes found the size of a walnut or even larger. In most cases they consist chiefly of calcium bilirubin with little or no biliverdin. Sometimes also small black or greenish-black, metallic-looking stones are found, which consist chiefly of bilifuscin along with biliverdin. Iron and copper seem to be regular constituents of pigment-stones. Manganese and zinc have also been found in a few cases. The pigment-stones are generally heavier than water.

The *cholesterin-stones*, whose size, form, color, and structure may vary greatly, are often lighter than water. The fractured surface is radiated, crystalline, and frequently shows crystalline, concentric layers. The cleavage fracture is waxy in appearance, and the fractured surface when rubbed by the finger-nail also becomes like wax. By rubbing against each other in the gall-bladder they often become faceted or take other remarkable shapes. Their surface is sometimes nearly white and waxlike, but generally their color is variable. They are sometimes smooth, in other cases they are rough or uneven. The quantity of cholesterin in the stones varies from 642 to 981 p. m. (RITTER[1]). The cholesterin-stones also sometimes contain variable amounts of lime-pigments, which may give them a very changeable appearance.

Cholesterin, $C_{27}H_{46}O$ (OBERMÜLLER), or, as ordinarily given, $C_{27}H_{44}O$ (MAUTHNER and SUIDA). By the action of concentrated sulphuric acid or phosphoric acid, and also in other ways, hydrocarbons are obtained, which are called *cholesteriline*, *cholesterone*, and *cholesterilene*. MAUTHNER and SUIDA,[2] who have closely studied these hydrocarbons, have been able to prepare a crystalline cholesteriline by heating cholesterin with anhydrous copper sulphate. The hydrocarbons stand, according to WEYL,[3] in close relationship to the terpene group, and the color reactions of cholesterin as well as the recent investigations on the constitution of this body seem to substantiate this view. Very painstaking and thorough investigations on the constitution of cholesterin have been made, of which we must especially mention those of MAUTHNER and SUIDA, WINDAUS and STEIN, DIELS and ABDERHALDEN.[4] Although these researches have not lead to positive conclusions, still we are justified in concluding that cholesterin probably consists of a complex of five hydrogenized rings, of which one contains a double bondage and another a secondary alcohol group. Several facts seem to make it probable that cholesterin stands close to the hydrogenized retene and hence is a complicated terpene. From this standpoint, the close relationship between cholesterin and cholic acid is of great interest.

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[1] Journ. de l'anat. et de la physiol. (Robin), 1872.

[2] Obermüller, Arch. f. (Anat. u.) Physiol., 1889, and Zeitschr. f. physiol. Chem., **15**; Mauthner and Suida, Wien. Sitzungsber., Math. Nat. Klasse, **103**, Abt. *2b*, which also contains the older literature.

[3] Arch. f. (Anat. u.) Physiol, 1886, p. 182.

[4] Mauthner and Suida, Monatshefte f. Chem., **15**, **17**, **24**; Windaus, "Über Cholesterin," Hab.-Schrift, Freiburg i. B., 1903, Ber. d. d. chem. Gesellsch., **36**, **37**, and **39**; with Stein, *ibid.*, **37**; Diels and Abderhalden, *ibid.*, **36**, **37**, and **39**; G. Stein, "Über Cholesterin," Inaug.-Dissert. Freiburg i. B., 1905.

On reduction of cholesterin, and also of the ketone, *cholesterone*, corresponding to cholesterin, by means of metallic sodium in amyl-alcohol solution, DIELS and ABDERHALDEN obtained two isomeric dihydrocholesterines, $C_{27}H_{48}O$, α- and *β-cholestanol*, of which the first seems to be identical with the dihydrocholesterin obtained by NEUBERG and RAUCHWERGER[1] by the action of sodium in amyl-alcohol solution. The identity of α-cholestanol with koprosterin, which will be mentioned below, is considered by NEUBERG as not improbable, while DIELS and ABDERHALDEN deny this.

Cholesterin occurs in small amounts in nearly all animal fluids and juices. It occurs only rarely in the urine, and then in very small quantities. It is also found in the different tissues and organs, especially abundant in the brain and the nervous system; further, in the yolk of the egg, in semen, in wool-fat (together with isocholesterin), and in sebum. It appears also in the contents of the intestine, in excrements, and in the meconium. It especially occurs pathologically in gall-stones, as well as in atheromatous cysts, in pus, in tuberculous masses, old transudates, cystic fluids, sputum, and tumors. It does not exist free in all cases; for example, it exists in part as fatty-acid esters in wool-fat, blood, lymph, brain, vernix caseosa, and epidermis formations. Several kinds of cholesterin, called *phytosterines*, have been found in the vegetable kingdom.

Cholesterin which has been crystallized from warm alcohol on cooling and that which is present in old transudates contains 1 molecule of water of crystallization, melts at 145° C., and forms colorless, transparent plates whose sides and angles frequently appear broken and whose acute angle is often 76° 30′ or 87° 30′. In large quantities it appears as a mass of white plates which shine like mother-of-pearl and have a greasy feeling.

Cholesterin is insoluble in water, dilute acids, and alkalies. It is neither dissolved nor changed by boiling caustic alkali. It is easily soluble in boiling alcohol and crystallizes on cooling. It dissolves readily in ether, chloroform, and benzene, and also in the volatile or fatty oils. It is dissolved to a slight extent by alkali salts of the bile-acids, better in the presence of oleic soap (GERARD[2]). The solutions in ether and chloroform are levorotatory.

Among the many compounds of cholesterin studied by OBERMÜLLER the propionic ester $C_2H_5.CO.O.C_{27}H_{45}$ is of special interest because of the behavior of the fused compound on cooling, and it is used in the detection of cholesterin. For the detection of cholesterin use is made of its reaction with concentrated sulphuric acid, which gives colored products.

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[1] Salkowski's Festschrift, 1904, and Neuberg, Ber. d. d. chem. Gesellsch., 39.

[2] Compt. rend. soc. biolog., 58.

If a mixture of five parts sulphuric acid and one part water acts on a cholesterin crystal, this crystal will show colored rings, first a bright carmine-red and then violet. This fact is employed in the microscopic detection of cholesterin. Another test, and one very good for the microscopical detection of cholesterin, consists in treating the crystals first with the above dilute acid and then with some iodine solution. The crystals will be gradually colored violet, bluish green, and a beautiful blue.

SALKOWSKI'S[1] *Reaction*. The cholesterin is dissolved in chloroform and then treated with an equal volume of concentrated sulphuric acid. The cholesterin solution becomes first bluish red, then gradually more violet-red, while the sulphuric acid appears dark red with a greenish fluorescence. If the chloroform solution is poured into a porcelain dish it becomes violet, then green, and finally yellow.

LIEBERMANN-BURCHARD'S[2] *Reaction*. Dissolve the cholesterin in about 2 c.c. chloroform and add first 10 drops of acetic anhydride and then concentrated sulphuric acid drop by drop. The color of the mixture will first be a beautiful red, then blue, and finally, if not too much cholesterin or sulphuric acid is present, a permanent green. In the presence of very little cholesterin the green color may appear immediately.

NEUBERG RAUCHWERGER'S[3] *Reaction*. With rhamnose, or better still with δ-methylfurfurol and concentrated sulphuric acid, an alcoholic solution of cholesterin gives a pink ring, or after mixing the liquids and cooling, a pink solution. On proper dilution an absorption-band can be seen just beginning before *E* and whose other side coincides with *b*. This reaction is of interest because it is also given by bile-acids, some camphor derivatives, abietinic acid, and a hydride of retene. For details of its performance, see original publication.

Pure, dry cholesterin when fused in a test-tube over a low flame with two or three drops of propionic anhydride yields a mass which on cooling is first violet, then blue, green, orange, carmine-red, and finally copper-red. It is best to re-fuse the mass on a glass rod and then to observe the rod on cooling, holding it against a dark background (OBERMÜLLER).

SCHIFF'S *Reaction*. If a little cholesterin is placed in a porcelain dish with the addition of a few drops of a mixture of 2 or 3 vols. of concentrated hydrochloric acid or sulphuric acid and 1 vol. of a rather dilute solution of ferric chloride and carefully evaporated to dryness over a small flame, a reddish-violet residue is first obtained and then a bluish-violet.

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[1] Pflüger's Arch., 6.

[2] C. Liebermann, Ber. d. deutsch. chem. Gesellsch., 18, 1804; H. Burchard, Beiträge zur Kenntnis der Cholesterine, Rostock, 1889.

[3] Salkowski's Festschrift, 1904.

If a small quantity of cholesterin is evaporated to dryness with a drop of concentrated nitric acid, one obtains a yellow spot which becomes deep orange-red with ammonia or caustic soda (not a characteristic reaction).

Koprosterin is the name given by Bondzynski to the cholesterin which was isolated by him from human fæces, although it was prepared earlier by Flint and designated as *stercorin*. It dissolves in cold, absolute alcohol and very readily in ether, chloroform, and benzene. It crystallizes in fine needles which melt at 95–96° C. (89–90° according to Hausmann),[1] and is dextrorotatory, $(\alpha)_D = +24°$. It gives the same color reactions as cholesterin, with the exception that it does not give a reaction with propionic anhydride. According to Bondzynski and Humnicki it is a dihydrocholesterin, with the formula $C_{27}H_{48}O$, which is formed in the human intestine by the reduction of ordinary cholesterin. These investigators have found another cholesterin, *hippokoprosterin*, with the formula $C_{27}H_{54}O$, in horses' fæces.

Isocholesterin is a cholesterin, so called by Schulze,[2] with the formula $C_{26}H_{43}OH$, which occurs in wool-fat and is therefore found to a great extent in so-called lanolin. It gives the Liebermann-Burchard reaction, but does not give Salkowski's reaction. It melts at 138–138.5° C.

Spongosterin is the name givenby Henze [3] to a cholesterin isolated by him from a silicious sponge. It is very similar to cholesterin but is not identical with it or with phytocholesterins. It gives the Liebermann-Burchard reaction as well as Salkowski's reaction, but the last test is not quite so beautiful a red. Obermüller's reaction is negative. Its specific rotation is $(\alpha)^{25}_{D} = 19.59°$.

The cholesterins belong to the so-called lipoids, which have been mentioned in previous chapters (V and VI) and are of the greatest importance as constituents of the outer envelope of erythrocytes and the cells in general. In this regard the cholesterin is of special interest for hæmolysis, in that, as shown by Ransom, it inhibits the hæmolytic action of saponin and hence it has a certain protective power in the animal body. This action of cholesterin, as found by Hausmann, is destroyed by replacing the hydroxyl groups. According to Madsen and Noguchi [4] the combination of cholesterin and saponin is a loose one.

The so-called cholesterin-stones are best employed in the preparation of cholesterin. The powder is first boiled with water and then repeatedly boiled with alcohol. The cholesterin which on cooling separates from the warm filtered solution is boiled with a solution of caustic potash in alcohol so as to saponify any fat. After the evaporation of the alcohol the cholesterin is extracted from the residue with ether, by which the soaps are not dissolved; filter, evaporate the ether, and purify the cholesterin by recrystallization from alcohol-ether. The cholesterin may be extracted with fat from tissues and fluids by first extracting with ether and then proceeding as suggested by RITTER.[1] The essential points in his method consist in saponifying the fat with sodium alcoholate, removing the alcohol by evaporating to dryness with NaCl, and finally extracting the dried, pulverized mass with ether. After evaporating the ether the residue is dissolved in as little alcohol as possible and the cholesterin precipitated by the addition of water. It is ordinarily easily detected in transudates and pathological formations by means of the microscope.

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[1] Bondzynski, Ber. d. deutsch. chem. Gesellsch., **29**; Bondzynski and Humnicki, Zeitschr. f. physiol. Chem., **22**; Flint, *ibid.*, **23**, and Amer. Journ. Med. Sciences, 1862; Müller, Zeitschr. f. physiol. Chem., **29**; Hausmann, Hofmeister's Beiträge, **6**.

[2] Ber. d. deutsch. chem. Gesellsch., **6**; Journal f. prakt. Chem. (N. F.), **25**; and Zeitschr. f. physiol. Chem., **14**, 522. See also E. Schulze and J. Barbieri, Journal f. prakt. Chem. (N. F.), **25**, 159. In regard to the formula for isocholesterin, see Darmstädter and Lifschütz, Ber. d. deutsch. chem. Gesellsch., **31**, and E. Schulze, *ibid.*, 1200.

[3] Zeitschr. f. physiol. Chem., **41**.

[4] Ransom, Deutsch. med. Wochenschr., 1901; Hausmann, Hofmeister's Beiträge, **6**; Madsen and Noguchi, Kgl. Dansk. Vidensk. Selskabs. Forh., 1904.

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[1] Zeitschr. f. physiol. Chem., **34**.

CHAPTER IX.

DIGESTION.

THE purpose of digestion is to separate those constituents of the food which serve as the nutriment of the body from those which are useless, and to separate each in such a form that it may be taken up by the blood from the alimentary canal and employed for various purposes in the organism. This demands not only mechanical, but also chemical action. The first action, which is essentially dependent upon the physical properties of the food, consists in a tearing, cutting, crushing, or grinding of the food, while the second serves chiefly in converting the nutritive bodies into a soluble and easily absorbed form, or in splitting them into simpler compounds for use in the animal syntheses. The solution of the nutritive bodies may take place in certain cases by the aid of water alone, but in most cases a chemical metamorphosis or cleavage is necessary; this is effected by means of the acid or alkaline fluids secreted by the glands. The study of the processes of digestion from a chemical standpoint must therefore begin with the digestive fluids, their qualitative and quantitative composition, as well as their action on the nutriments and foods.

I. The Salivary Glands and the Saliva.

The **salivary glands** are partly *albuminous glands* (as the parotid in man and mammals and the submaxillary in rabbits), partly *mucous glands* (as some of the small glands in the buccal cavity and the sublingual and submaxillary glands of many animals), and partly *mixed glands* (as the submaxillary gland in man). The alveoli of the albuminous glands contain cells which are rich in proteid but which contain no mucin. The alveoli of the mucin-glands contain cells rich in mucin but poor in proteid. Cells arranged in different ways, but rich in proteids, also occur in the submaxillary and sublingual glands. According to the analyses of OIDTMANN[1] the salivary glands of a dog contain 790 p. m. water, 200 p. m. organic and 10 p. m.

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[1] Cit. from v. Gorup-Besanez, Lehrbuch d. physiol. Chem., 4. Aufl., 732. The figures there given amount to 1010 parts instead of 1000 parts.

inorganic solids. Among the solids we find *mucin, proteids, nucleoproteids, nuclein, enzymes* and their *zymogens,* besides *extractive bodies, leucine, xanthine bodies,* and *mineral substances.*

The occurrence of a mucinogen has not been proved. On the complete removal of all mucin E. HOLMGREN [1] found no mucinogen in the submaxillary gland of the ox, but a mucin-like gluconucleoproteid.

The **saliva** is a mixture of the secretion of the above-mentioned groups of glands; therefore it is proper that a study be made of each of the different secretions by itself and then of the mixed saliva.

The **submaxillary saliva** in man may be easily collected by introducing a canula through the papillary opening into Wharton's duct.

The submaxillary saliva has not always the same composition or properties; this depends essentially, as shown by experiments on animals, upon the conditions under which the secretion takes place. That is to say, the secretion is partly dependent on the cerebral system, through the facial fibres in the chorda tympani, and partly on the sympathetic nervous system, through the fibres entering the vessels in the gland. In consequence of this dependence the two distinct varieties of submaxillary secretion are distinguished as *chorda-* and *sympathetic* saliva. A third kind of saliva, the so-called *paralytic saliva,* is secreted after poisoning with curare or after the severing of the glandular nerves.

The difference between chorda- and sympathetic saliva (in dogs) consists chiefly in their quantitative constitution; the less abundant sympathetic saliva is more viscous and richer in solids, especially in mucin, than the more abundant chorda-saliva. The specific gravity of the chorda-saliva of the dog is 1.0039–1.0056, and contains 12–14 p. m. solids (ECKHARD [2]). The sympathetic has a specific gravity of 1.0075–1.018, with 16–28 p. m. solids. The freezing-point of the chorda-saliva obtained from dogs on electric stimulation varies, according to NOLF,[3] between $\Delta = -0.193°$ and $-0.396°$, with a content of 3.3–6.5 p. m. salts and 4.1–11.5 p. m. organic substances. The osmotic pressure is on an average a little higher than one half the osmotic pressure of the blood-serum. The spontaneously secreted submaxillary saliva is ordinarily somewhat diluted. Other investigators, such as ASHER and CUTTER,[4] have also found that the osmotic pressure of the submaxillary saliva is considerably lower than that of the blood. The gases of the chorda-saliva have been investigated by PFLÜGER.[5] He found 0.5–0.8 per cent oxygen, 0.9–1 per cent nitrogen, and 64.73–85.13

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[1] Upsala Läkaref. Förh. (N. F.), **2**; also Maly's Jahresber., **27**.

[2] Cited from Kühne's Lehrb. d. physiol. Chem., 7

See Maly's Jahresber., **31**, 494.

[4] Zeitschr. f. Biologie, **40**.

[5] Pflüger's Arch., **1**.

per cent carbon dioxide—all results calculated at 0° C. and 760 mm. pressure. The greater part of the carbon dioxide was chemically combined.

The two kinds of submaxillary secretion just named have not thus far been separately studied in man. The secretion may be excited by an emotion, by mastication, and by irritating the mucous membrane of the mouth, especially with acid-tasting substances. The submaxillary saliva in man is ordinarily clear, rather thin, a little ropy, and froths easily. Its reaction is alkaline towards litmus. The specific gravity is 1.002–1.003, and it contains 3.6–4.5 p. m. solids.[1] As organic constituents are found mucin, traces of proteid and diastatic enzyme, which latter is absent in several species of animals. The inorganic bodies are alkali chlorides, sodium and magnesium phosphates, and bicarbonates of the alkalies and calcium. Potassium sulphocyanide occurs in this saliva.

The Sublingual Saliva. The secretion of this saliva is also influenced by the cerebral and the sympathetic nervous system. The chorda-saliva, which is secreted only to a small extent, contains numerous salivary corpuscles, but is otherwise transparent and very ropy. Its reaction is alkaline, and it contains, according to HEIDENHAIN,[2] 27.5 p. m. solids (in dogs).

The sublingual secretion in man is clear, mucilaginous, more alkaline than the submaxillary saliva, and contains mucin, diastatic enzyme, and potassium sulphocyanide.

Buccal mucus can be obtained pure from animals only by the method suggested by BIDDER and SCHMIDT, which consists in tying the exit to all the large salivary glands and cutting off their secretion from the mouth. The quantity of liquid secreted under these circumstances (in dogs) was so very small that the investigators named were able to collect only 2 grams of buccal mucus in the course of one hour. It is a thick, ropy, sticky liquid containing mucin; it is rich in form-elements, above all in flat epithelium-cells, mucous cells, and salivary corpuscles. The quantity of solids in the buccal mucus of the dog is, according to BIDDER and SCHMIDT,[3] 9.98 p. m.

Parotid Saliva. The secretion of this saliva is also partly dependent on the cerebral nervous system (n. glossopharyngeus) and partly on the sympathetic. The secretion may be excited by emotions and by irritation of the glandular nerves, either directly (in animals) or reflexly, by mechanical or chemical irritation of the mucous membrane of the mouth. Among the chemical irritants the acids take first place. Mastication also exercises a strong influence upon the secretion of parotid saliva, which is specially marked in certain herbivora.

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[1] See Maly, "Chemie der Verdauungssäfte und der Verdauung," in Hermann's Handb., 5, part II, 18. This article contains also the pertinent literature.

[2] Studien d. physiol. Instituts zu Breslau, Heft 4.

[3] Die Verdauungssäfte und der Stoffwechsel (Mitau and Leipzig, 1852), 5.

Human parotid saliva may be readily collected by the introduction of a canula into STENSON's duct. This saliva is thin, less alkaline than the submaxillary saliva (the first drops are sometimes neutral or acid), without special odor or taste. It contains a little proteid but no mucin, which is to be expected from the construction of the gland. It also contains a diastatic enzyme, which, however, is absent in many animals. The quantity of solids varies between 5 and 16 p. m. The specific gravity is 1.003–1.012. Potassium sulphocyanide seems to be present, though it is not a constant constituent. KÜLZ[1] found a maximum of 1.46 per cent oxygen, 3.8 per cent nitrogen, and in all 66.7 per cent carbon dioxide in human parotid saliva. The quantity of firmly combined carbon dioxide was 62 per cent.

The quantity and composition of the saliva from the mucin glands as well as from the albuminous glands, as PAWLOW's[2] school has shown, is greatly dependent in dogs upon the psychical excitement, but also upon the kind of substances introduced into the mouth, and an adaptation of the glands for various mechanical and chemical irritants is found to occur. Under the influence of hard and dry food the glands secrete abundance of saliva, while with food rich in water the secretion is considerably less and accommodates itself according to the quantity of water in the food. Milk is an exception to this rule, as it causes a more abundant secretion of saliva than meat. This is of importance in digestion of milk, as in the stomach the mixture of milk and saliva does not coagulate to a compact mass but separates in a finely divided, readily digestible condition. By the action of strong chemical bodies the saliva is secreted in proportion to the strength of the irritant. The irritants are thereby diluted and the mouth washed out at the same time. The partaking of acids brings about the secretion of a thin saliva, poor in mucin, in quantities sufficient to neutarlize the acid, while on the introduction of food the glands secrete a saliva rich in mucin and diastatic enzymes.

The mixed buccal saliva in man is a colorless, faintly opalescent, slightly ropy, easily frothing liquid without special odor or taste. It is made turbid by epithelium-cells, mucous and salivary corpuscles, and often by food residues. Like the submaxillary and parotid saliva, on exposure to the air it becomes covered with an incrustation consisting of calcium carbonate and a small quantity of an organic substance, or it gradually becomes cloudy. Its reaction is generally alkaline to litmus. The degree of alkalinity varies considerably not only in different individuals but also in the same individual during different parts of the day, so that it is difficult to state the average alkalinity. According to CHITTENDEN and ELY it corresponds to the alkalinity of 0.8 p. m. Na_2CO_3 solution, or to 0.2 p. m. solution according to COHN. According to FOA the actual alkalinity (OH-ion concentration) is always considerably less than that found by titration, and the reaction determined electrometrically is very nearly neutral. The reaction may also be acid, as found by STICKER to be the case some time after a meal, but this is not true at least for all individuals. The specific gravity varies between 1.002 and 1.008, and the quantity of solids between 5 and 10 p. m. According to COHN[1], $\Delta = -0.20°$ on an average and the amount of NaCl is 1.6 p. m. The solids, irrespective of the form-constituents mentioned, consist of *protein, mucin, oxidases,*[2] two enzymes, *ptyalin* and *maltase*, and *mineral bodies* It is also claimed that *urea* is a normal constituent of the saliva. The mineral bodies are alkali chlorides, bicarbonates of the alkalies and calcium, phosphates, and traces of sulphates, nitrites, ammonia, and sulphocyanides, which latter average about 0.1 p. m. (MUNK and others). Smaller quantities, 0.03–0.04 p. m., are found in the saliva of non-smokers (SCHNEIDER and KRÜGER), while from ordinary smokers the quantity of sulphocyanides may rise to 0.2 p. m. (FLECKSEDER[3]).

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[1] Zeitschr. f. Biologie, **23**.

[2] Arch. internation. de Physiol., **1**, 1904. See also Neilson and Terry, Amer. Journ. of Physiol., **15**. Somewhat contradictory statements in regard to the accommodation of the secretion of the glands to requirements (in man) can be found in Zebrovski, Pflüger's Arch., **110**.

Sulphocyanides, which, although not constant, occur in the saliva of man and certain animals, may be easily detected by acidifying the saliva with hydrochloric acid and treating with a very dilute solution of ferric chloride. As control, especially in the presence of very small quantities, it is best to compare the test with another test-tube containing an equal amount of acidulated water and ferric chloride. Other methods have been suggested by GSCHEIDLEN, SOLERA, and GANASSINI. The quantitative estimation can be done according to MUNK's[4] method.

Ptyalin, or salivary diastase, is the amylolytic enzyme of the saliva. This enzyme is found in human saliva,[5] but not in that of all animals, especially not in the typical carnivora. It occurs not only in adults, but also in new-born infants. In opposition to ZWEIFEL's views, BERGER[1] claims that it is present not only in the parotid gland of children, but also in the mucin gland.

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[1] Chittenden and Ely, Amer. Chem. Journ., 4, 1883; Chittenden and Richards, Amer. Journ. of Physiol., 1; Foa, Compt. rend. soc. biolog., 58; Sticker, cited from Centralbl. f. Physiol., 3, 237; Cohn, Deutsch. med. Wochenschr., 1900.

[2] Bogdanow-Beresowski, cited from Biochem. Centralbl., 2, 653.

[3] Munk, Virchow's Arch., 69; Schneider, Amer. Journ. of Physiol., 5; Krüger, Zeitschr. f. Biologie, 37; Fleckseder, Centralbl. f. innere Med., 1905. In regard to the variation in the amount of various constituents in saliva see Fleckseder, l. c., and Tezner, Arch. internation. de Physiol., 2.

[4] Gscheidlen, Maly's Jahresber., 4; Solera, see *ibid.*, 7 and 8; Munk, Virchow's Arch., 69; Ganassini, Biochem. Centralbl., 2, p. 361.

[5] In regard to the variation in the quantity of ptyalin in human saliva see Hofbauer, Centralbl. f. Physiol., 10, and Chittenden and Richards, Amer. Journ. of Physiol., 1; Schüle, Maly's Jahresber., 29; Tezner, l. c.

According to H. GOLDSCHMIDT[2] the saliva (parotid saliva) of the horse does not contain ptyalin, but a zymogen of the same, while in other animals and man the ptyalin is formed from the zymogen during secretion. In horses the zymogen is transformed into ptyalin during mastication, and bacteria seem to give the impulse to this change. During precipitation with alcohol the zymogen is changed into ptyalin.

Ptyalin has not been isolated in a pure form up to the present time. It can be obtained purest by COHNHEIM'S[3] method, which consists in carrying the enzyme down mechanically with a calcium-phosphate precipitate and washing the precipitate with water, which dissolves the ptyalin, and from which it can be obtained by precipitating with alcohol. For the study or demonstration of the action of ptyalin one employs a watery or glycerine extract of the salivary glands, or simply the saliva itself.

Ptyalin, like other enzymes, is characterized by its action. This consists in converting starch into dextrins and sugar. The process going on in this conversion may be described as follows: In the first stages soluble starch or *amidulin* is formed. From this amidulin, erythrodextrin and sugar are produced by hydrolytic cleavage. The erythrodextrin then splits into α-achroodextrin and sugar. From this achroodextrin by splitting β-achroodextrin and sugar are formed, and finally this β-achroodextrin splits into sugar and γ-achroodextrin. Other investigators explain this process in another manner (see Chapter III), hence the exact procedure is not completely clear. Still the results are positive as to the sugar produced in this process. For a long time it was considered that dextrose was the sugar formed from starch and glycogen, but SEEGEN and O. NASSE have shown that this is not true. MUSCULUS and v. MERING have shown that the sugar formed by the action of saliva, amylopsin, and diastase upon starch and glycogen is for the most part maltose. This has been substantiated by BROWN and HERON. E. KÜLZ and J. VOGEL[4] have also demonstrated that in the saccharification of starch and glycogen, isomaltose, maltose, and some dextrose are formed, the varying quantities depending upon the amount of ferment and the length of its action. The formation of dextrose is claimed by **Tebb**, **Röhmann**, and **Hamburger**[1] to be only a product of the inversion of the maltose by the maltase.

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[1] Zweifel, Untersuchungen über den Verdauungsapparat der Neugeborenen (Berlin, 1874); Berger, see Maly's Jahresber., **30**, 399.

[2] Zeitschr. f. physiol. Chem., **10**.

[3] Virchow's Arch., **28**.

[4] Seegen, Centralbl. f. d. med. Wissensch., 1876, and Pflüger's Arch., **19**; Nasse *ibid.*, **14**; Musculus and v. Mering, Zeitschr. f. physiol. Chem., **2**; Brown and Heron, Liebig's Annal., **199** and **204**; Külz and Vogel, Zeitschr. f. Biologie, **31**

The action of ptyalin in various *reactions* has been the subject of numerous investigations.[2] Natural alkaline saliva is very active, but it is not so active as when made neutral. It may be still more active under certain circumstances in faintly acid reaction, and according to **Chittenden** and **Smith** it acts better when enough hydrochloric acid is added to saturate the proteins present than when only neutralized. When the acid-combined protein exceeds a certain amount, then the diastatic action is diminished. The addition of alkali to the saliva decreases its diastatic action; on neutralizing the alkali with acid or carbon dioxide the retarding or preventive action of the alkali is arrested. According to **Schierbeck**, carbon dioxide has an accelerating action in neutral liquids, while **Ebstein** claims that it has, as a rule, a retarding action. Organic as well as inorganic acids, when added in sufficient quantity, may stop the diastatic action entirely. The degree of acidity necessary in this case is not always the same for a certain acid, but is dependent upon the quantity of ferment. The same degree of acidity in the presence of large amounts of ferment has a weaker action than in the presence of smaller quantities. Hydrochloric acid is of special physiological interest in this regard, for it prevents the formation of sugar even in very small amounts (0.03 p. m.). Hydrochloric acid has not only the property of preventing the formation of sugar, but, as shown by **Langley**, **Nylén**, and others, may entirely destroy the enzyme. This is important in regard to the physiological significance of the saliva. That boiled starch (paste) is quickly, and unboiled starch only slowly, converted into sugar is also of interest. Various kinds of unboiled starch are converted with different degrees of rapidity.

Several series of investigations have been made upon the velocity with which ptyalin acts, and as in testing enzyme action in general, the experimenters have not made use of the different times required to produce equal chemical changes as a measure of the velocity, but have taken the quantities of substance changed in equal times. Although the results are somewhat divergent it is possible to deduce the following from them. The velocity increases, at least under conditions otherwise favorable, with the *amount of enzyme* and with an increasing *temperature* to a little above 40° C.

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[1] Tebb, Journ. of Physiol., **15**; Röhmann, Ber. d. deutsch. chem. Gesellsch., **27**; Hamburger, Pflüger's Arch., **60**.

[2] See Hammarsten, Maly's Jahresber., **1**; Chittenden and Griswold, Amer. Chem. Journ., **3**; Langley, Journal of Physiol., **3**; Nylén, Maly's Jahresber., **12**, 241; Chittenden and Ely, Amer. Chem. Journ., **4**; Langley and Eves, Journal of Physiol., **4**; Chittenden and Smith, Yale College Studies, **1**, 1885, 1; Schlesinger, Virchow's Arch., **125**; Schierbeck, Skand. Arch. f. Physiol., **3**; Ebstein and C. Schulze, Virchow's Arch. **134**; Kübel, Pflüger's Arch., **76**.

Foreign substances, such as metallic salts,[1] have different effects. Certain salts even in small quantities completely arrest the action; for example, $HgCl_2$ accomplishes this result completely by the presence of only 0.05 p. m Other salts, such as magnesium sulphate, in small quantities (0.25 p. m.) accelerate, and in larger quantities (5 p. m.) check the action. The presence of peptone has an accelerating action on the sugar formation (CHITTENDEN and SMITH and others). The *accumulation of the products of the amylolytic decomposition* also checks the action of the saliva. This has been shown by special experiments made by SH. LEA.[2] He made parallel experiments with digestions in test-tubes and in dialyzers, and found on the removal of the products of the amylolytic decomposition by dialysis that the formation of sugar took place sooner, but also that considerably more maltose and less dextrin were formed.

To show the action of saliva or ptyalin on starch the three ordinary tests for dextrose may be used, namely, MOORE'S or TROMMER'S test or the *bismuth test* (see Chapter XV). It is also necessary, as a control, to first test the starch-paste and the saliva for the presence of dextrose. The steps in the transformation of starch into amidulin, erythrodextrin, and achroodextrin may be shown by testing with iodine.

Maltase occurs in saliva to only a slight extent. It converts maltose into dextrose. According to STICKER [3] saliva also has the power of splitting sulphuretted hydrogen from the sulphur oils of radishes, onions, and certain other vegetables.

The *quantitative composition* of the mixed saliva must vary considerably, not only because of individual differences, but also because under varying conditions there may be an unequal division of the secretion from the different glands. We give opposite a few analyses of human saliva as examples of its composition. The results are in parts per 1000.

HAMMERBACHER found in 1000 parts of the ash from human saliva: potash 457.2, soda 95.9, iron oxide 50.11, magnesia 1.55, sulphuric anhydride (SO_3) 63.8, phosphoric anhydride (P_2O_5) 188.48, and chlorine 183.52.

The quantity of saliva secreted during twenty-four hours cannot be exactly determined, but has been calculated by BIDDER and SCHMIDT to be 1400–1500 grams. The most abundant secretion occurs during meal-times. According to the calculations and determinations of TUCZEK [4], in man 1 gram of gland yields 13 grams of secretion in the course of one hour during mastication. These figures correspond fairly well with those representing the average secretion from 1 gram of gland in animals, namely, 14.2 grams in the horse and 8 grams in oxen. The quantity of secretion per hour may be 8 to 14 times greater than the entire mass of glands, and there is probably no gland in the entire body, so far as is known at present—the kidneys not excepted—whose ability of secretion under physiological conditions equals that of the salivary glands. A remarkably abundant secretion of saliva is induced by pilocarpine, while atropine, on the contrary, prevents it.

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[1] See O. Nasse, Pflüger's Arch., **11**, and Chittenden and Painter, Yale College Studies, **1**, 1885, 52; Kübel, Pflüger's Arch., **76**.

[2] Journ. of Physiol., **11**.

[3] Münch. med. Wochenschr., **43**.

[4] Bidder and Schmidt, l. c., 13; Tuczek, Zeitschr. f. Biologie, **12**.

	BERZELIUS.	JACUBOWITSCH.	FRERICHS.	TIEDEMANN and GMELIN.	HERTER.	LEHMANN.	HAMMERBACHER.[1]
Water	992.9	995.16	994.1	988.3	994.7	....	994.2
Solids	7.1	4.84	5.9	11.7	5.3	3.5-8.4 in filtered saliva.	5.8
Mucus and epithelium	1.4	1.62	2.13	....	....	....	2.2
Soluble organic substances (Ptyalin of early investigators.)	3.8	1.34	1.42	....	3.27	....	1.4
Sulphocyanides	....	0.06	0.10	....	....	0.064 to 0.090	0.04
Salts	1.9	1.82	2.19	....	1.30	....	2.2

That the secretion of saliva, even if we do not consider such substances as ptyalin, mucin, and the like, is not a process of filtration, follows from many reasons, especially the following: The salivary glands have, a specific property of eliminating certain substances, such as potassium salts (SALKOWSKI [2]), iodine, and bromine compounds, but not others, for example, iron compounds and dextrose. It is also noticeable that the saliva is richer in solids when it is eliminated quickly by gradually increased stimulation, and in larger quantities than when the secretion is slower and less abundant (HEIDENHAIN). The amount of salts increases also to a certain degree by an increasing rapidity of elimination (HEIDENHAIN, WERTHER, LANGLEY and FLETCHER, NOVI [3]).

Like the secretion processes in general, the secretion of saliva is closely connected with the processes in the cells. The chemical processes going on in these cells during secretion are still unknown.

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[1] Zeitschr. f. physiol. Chem., 5. The other analyses are cited from Maly, Chemie der Verdauungssäfte, Hermann's Handbuch d. Physiol., 5, part II, 14.

[2] Virchow's Arch., 53.

[3] Heidenhain, Pflüger's Arch., 17; Werther, *ibid.*, 38; Langley and Fletcher, Proc. Roy. Soc., 45, and especially Phil. Trans. Roy. Soc. London, 180; Novi, Arch. f. (Anat. u.) Phsyiol., 1888.

The Physiological Importance of the Saliva. The quantity of water in the saliva renders possible the action of certain bodies on the organs of taste, and it also serves as a solvent for a part of the nutritive substances. The importance of the saliva in mastication is especially marked in herbivora, and there is no question as to its importance in facilitating the act of swallowing. The saliva containing mucin is especially important in this regard, and PAWLOW's school has shown that the secretion also regulates itself in this regard. The saliva is also of importance, as it serves in washing out the mouth and thereby acts as a protection against destructive substances or bodies foreign to the mouth. The power of converting starch into sugar is not inherent in the saliva of all animals, and even when it possesses this property the intensity varies in different animals. In man, whose saliva forms sugar rapidly, a production of sugar from (boiled) starch undoubtedly takes place in the mouth, but how far this action proceeds after the morsel has entered the stomach depends upon the rapidity with which the acid gastric juice mixes with the swallowed food, and also upon the relative amounts of the gastric juice and food in the stomach. The large quantity of water which is swallowed with the saliva must be absorbed and pass into the blood, and it must in this way go through an intermediate circulation in the organism. Thus the organism possesses in the saliva an active medium by which a constant stream, conveying the dissolved and finely divided bodies, passes into the blood from the intestinal canal during digestion.

Salivary Concrements. The so-called tartar is yellow, gray, yellowish-gray, brown or black, and has a stratified structure. It may contain more than 200 p. m. organic substances, which consist of mucin, epithelium, and LEPTOTHRIX-CHAINS. The chief part of the inorganic constituents consists of calcium carbonate and phosphate. The salivary calculi may vary in size from that of a small grain to that of a pea or still larger (a salivary calculus has been found weighing 18.6 grams), and they contain variable quantities of organic substances (50–380 p. m.), which remain on extracting the calculus with hydrochloric acid. The chief inorganic constituent is calcium carbonate.

II. The Glands of the Mucous Membrane of the Stomach, and the Gastric Juice.

Since long ago the glands of the mucous coat of the stomach have been divided into two distinct classes. Those which occur in the greatest abundance and which have the greatest size in the fundus are called *fundus glands*, also rennin or pepsin glands, and the others which occur only in the neighborhood of the pylorus have received the name of *pyloric glands*, sometimes also, though incorrectly, called *mucous glands*. The division of these two forms of glands in the mucous membrane of the stomach is essentially different in various animals. The mucous coating of the stomach is covered throughout with a layer of columnar epithelium, which is generally considered as consisting of goblet cells that produce mucus by a metamorphosis of the protoplasm.

The **fundus glands** contain two kinds of cells: ADELOMORPHIC or chief cells, and DELOMORPHIC or PARIETAL cells, the latter formerly called RENNIN or pepsin cells. Both kinds consist of protoplasm rich in proteins; but their relationship to coloring-matters seems to show that the protein substances of both are not identical. The nucleus must consist chiefly of nuclein. Besides the above-mentioned constituents, the fundus glands contain as more specific constituents several enzymes or their zymogens, besides a little fat and cholesterin.

The **pyloric glands** contain cells which are generally considered as related to the above-mentioned chief cells of the fundus glands. As these glands were formerly thought to contain a larger quantity of mucin, they were also called mucous glands. According to HEIDENHAIN, independent of the columnar epithelium of the excretory ducts they take no part worthy of mention in the formation of mucus, which according to his views is effected by the epithelium covering the mucous membrane. The pyloric glands also seem to contain the *zymogens* referred to above. Alkali chlorides, alkali phosphates, and calcium phosphates are found in the mucous coating of the stomach.

LIEBERMANN[1] has obtained an acid-reacting residue on digesting the mucosa of the stomach with pepsin-hydrochloric acid, which strangely enough contained no nuclein, but only a protein containing lecithin, called lecithalbumin. To this lecithalbumin he ascribes a great importance in the secretion of hydrochloric acid.

The Gastric Juice. The observations of HELM and BEAUMONT on persons with gastric fistula led to the suggestion that gastric fistulas be made on animals, and this operation was first performed by BASSOW[2] in 1842 on a dog. VERNEUIL performed the same on a man in 1876 with successful results. PAWLOW[3] has recently improved the surgery of gastric fistula and has added much to the study of gastric secretion.

The secretion of gastric juice is not continuous, at least in man and in the mammals experimented upon. It only occurs under psychic influence, and also by stimulation of the mucous membrane of the stomach or the intestine. The most exhaustive researches on the secretion of gastric juice (in dogs) have been made by PAWLOW and his pupils.

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[1] Pflüger's Arch., 50.

[2] Helm, Zwei Krankengeschichten, Wien, 1803, cit. from Hermann's Handbuch, 5. part II, 39; Beaumont, "The Physiology of Digestion," 1833; Bassow, Bull. de la soc. des natur. de Moscou, 16, cit. from Maly in Hermann's Handbuch, 5, 38; Verneuil, see Ch. Richet, "Du suc gastrique chez l'homme," etc. (Paris, 1878), 158.

[3] Pawlow, Die Arbeit der Verdauungsdrüsen (Wiesbaden, 1898), where the works of his pupils are also mentioned. See also Ergebnisse der Physiologie, 1, Abt. 1.

In order to obtain gastric juice free from saliva and food residues they arranged besides a gastric fistula also an œsophageal fistula from which the swallowed food could be withdrawn with the saliva without entering the stomach, and in this manner an apparent feeding was possible. In this way it was possible to study the influence of psychical moments on one side and the direct action of food on the mucous membrane on the other. After a method suggested by HEIDENHAIN and later improved by PAWLOW and CHIGIN, they have succeeded in preparing a blind sac by partial dissection of the fundus part of the stomach, and the secretion processes could be studied in this sac while the digestion in the other parts of the stomach was going on. In this way they were able to study the action of different foods on the secretion.

The most essential results of the investigations of PAWLOW and his pupils are as follows: Mechanical stimulation of the mucosa does not produce any secretion. Chemical and mechanical irritations of the mucous membrane of the mouth cause no reflex excitation of the secretory nerves of the stomach. There are two moments which cause a secretion, namely, the psychical moment—the passionate desire for food and the sensation of satisfaction and pleasure on partaking it—and the chemical moment, the action of certain chemical substances on the mucous membrane of the stomach. The first moment is the most important. The secretion occurring under its influence by the vagus fibres appears earlier than that produced by chemical irritants, but only after an interval of at least 4½ minutes. This secretion is more abundant but less continuous than the "chemical." It yields a more acid and active juice than the latter. As chemical excitants which cause a secretion reflexively through the stomach mucosa we include only water and certain unknown extractive substances contained in meat and meat extracts, in impure peptone, and also, it seems, in milk. According to HERZEN and RADZIKOWSKI[1] alcohol is also a strong agent in producing a flow of juice. Carbonated alkalies have a preventive instead of an accelerating action on secretion. Bitter substances partaken of in small amounts a certain time before a meal increase the secretion, while larger amounts have a retarding action (BORISSOW, STRASHESKO[2]). Fats have a retarding action on the appearance of secretion and diminish the quantity of juice secreted as well as the amount of enzyme. The substances, such as egg-albumin, which act as chemical stimulants cannot be digested by the "psychical" secretion, but may perhaps cause a chemical secretion by their decomposition products.

The quantity of juice secreted during digestion is proportional to the quantity of food, and the secretion of gastric juice may also be influenced by the kind of food. This action of various foods—meat, bread, and milk—may be arranged in progressive series as follows:

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[1] Pflüger's Arch., 84, 513.

[2] Borissow, Arch. f. exp. Path. u. Pharm., 51; Strashesko, see Biochem. Centralbl., 4, 148.

	Acidity.	Digestive Activity.	Duration of Secretion.
1.	Meat.	Bread.	Bread.
2.	Milk.	Meat.	Meat.
3.	Bread.	Milk.	Milk.

The acidity is greatest with a meat diet and lowest with bread; the quantity of enzyme is, on the contrary, highest with a bread diet and lowest with milk.

The secretion in the stomach may also be influenced by the small intestine, and in this way, as shown by the investigations of Pawlow and his pupils, the fats have a retarding action upon the secretion of juice and upon digestion by acting reflexly upon the duodenal mucosa. In dogs on feeding fat (oil) with food containing starch, the secretion of gastric juice remains reduced during the entire period of feeding, and fat in connection with protein food has a similar action, with the exception that in this case the retarding action is observed only in the first hours of digestion. According to Piontkowski[1] the oil-soaps differ from the neutral fats by having a strong action on the flow of juice, and this is the reason why about 5 to 6 hours after a meal with fat the secretion of juice is stopped, as just at this time the soaps are being formed. According to Frouin the food in the intestine produces a secretion of gastric juice which continues after the action of the psychic moment has ceased. Leconte[2] arrived at similar results, and he ascribes less importance to the chemical secretion as compared with the psychic secretion than Pawlow does.

Lönnquist[3] has made observations upon dogs as to the dependence of the secretion of gastric juice upon the presence of food or substances causing flow in the stomach or in the intestine alone, or simultaneously in both, with the exclusion of the psychical influence. For this purpose he experimented with the stomach, isolated according to Heidenhain-Pawlow, as well as with fistulæ in the stomach and intestine, and besides this he also separated the stomach and intestine from each other by means of a membrane between the pylorus and the intestine produced by operative means. An abundant secretion of juice was produced in the stomach isolated from the intestine by water, alcohol, meat, and meat extracts, and by the digestive products of egg-albumin. Dilute hydrochloric acid (0.1–0.5 per cent) or natural gastric juice caused only a faint secretion. Dilute sodium-chloride or soda solutions (0.25–0.50 per cent) acted somewhat like water; stronger soda solutions (1–1.5 per cent) produced a much greater secretion of juice. Water or salt solution in the duodenum was without action. Liquid fat had (reflexly) a strong retarding action, and soda solutions, in the same manner, had a weaker retarding action. Water as well as alcohol was absorbed from the stomach, and the alcohol acted for the first half hour as a strong excitant for the flow of juice.

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[1] See Biochem. Centralbl., 3, 660.

[2] Frouin, Compt. rend. soc. biol., 53; Leçonte, La Cellule, 17.

[3] "Bidrag tils Kännedomen om magsaftafsöndringen," Akademisk afhandling. Helsingfors, 1906.

We know very little positively in regard to the gastric secretion in man. According to the older statements the irritants may be mechanical, thermic, and chemical. Among the chemical excitants we include alcohol and ether, which in too great a concentration bring about no physiological secretion, rather the transudation of a neutral or faintly alkaline fluid. Certain acids, such as carbonic acid, neutral salts, meat extracts, spices, and other bodies also belong to this group. The statements on this subject are unfortunately very uncertain and contradictory, still there is no doubt that in man, at least, alcohol and meat extracts may be active in causing secretion.

The question as to how far the observations made by PAWLOW and his school can be applied to man is of special interest; still we have only very few statements on this point at the present time. HORNBORG,[1] who recently studied a case of gastric fistula with œsophageal stricture in a boy, could not observe any special influence of the psychic excitement. The chewing of indifferent or bad tasting bodies had no action, while on the contrary the chewing of bodies with a pleasant taste produced a more or less abundant secretion. UMBER[2] observed in only one instance, in a case of a man after gastrotomy, an insignificant truly psychic secretion of gastric juice; chewing of an indifferent body or of chewing-tobacco brought about no secretion. After an apparent feeding with meat, with a latent period of 3 minutes, a secretion of gastric juice rich in HCl and enzymes occurred. Contrary to the behavior in dogs, the juice secreted after chewing bread was richer in acid than after chewing meat; the quantity was on the contrary less. UMBER also observed that after the introduction of a food enema into the rectum a secretion of gastric juice was produced by reflex action. CADE and LATARJET[3] have made observations on a girl twenty years old who had a blind sac formed by constriction, which was analogous to PAWLOW's "little stomach." The juice which flowed from the fistula opening of this blind sac was at least not always normal, judging from the amount of acid and by its action. In this person a purely psychic secretion was unquestionably observed by a continuous recollection of a pleasant sensation of taste, although it was not especially strong.

From these observations of HORNBORG and UMBER, as well as from some older observations of SCHÜLE, TROLLER, RIEGEL, and SCHEUER,[1] we conclude that in man the psychic secretion is much less than that produced by the introduction of food or bodies having a pleasant taste. That the preparation of the food in the mouth has an essential influence upon the secretion is proven without doubt, but we are not united as to how this action takes place. Certain experimenters consider the secreted and swallowed saliva as the most essential factor in this action, while others believe that the act of chewing, and still others that the chemical action and the sense of taste, are the most important.

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[1] Hornborg, "Bidrag till kännedomen om magsaftafsöndringen hos människan" Inaug.-Dissert. Helsingfors, 1903.

[2] Berlin. klin. Wochenschr., 1905.

[3] Compt. rend. soc. biolog., 57.

The Qualitative and Quantitative Composition of the Gastric Juice. The human gastric juice, which can seldom be obtained pure and free from residues of the food or from mucus and saliva, is a clear, or only very faintly cloudy, and in man nearly colorless fluid of an insipid, acid taste and strong acid reaction. It contains, as form-elements, *glandular cells* or their *nuclei*, *mucus-corpuscles*, and more or less changed *columnar epithelium.*

The acid reaction of the gastric juice depends on the presence of free acid, which, as has been learned from the investigations of C. SCHMIDT, RICHET, and others, consists, when the gastric juice is pure and free from particles of food, chiefly or in large part of *hydrochloric acid.* CONTEJEAN[2] has regularly found traces of lactic acid in the pure gastric juice of fasting dogs. After partaking of food, especially after a meal rich in carbohydrates, lactic acid occurs abundantly, and sometimes acetic and butyric acids. In new-born dogs the acid of the stomach is lactic acid, according to GMELIN.[3] The quantity of free hydrochloric acid in the gastric juice is, according to PAWLOW and his pupils, in dogs 5–6 p. m., and in cats an average of 5.20 p. m. HCl. In man the acidity has been found to vary considerably, but it is generally calculated as 2–3 p. m. HCl. According to VERHAEGEN'S researches there is no doubt that pure human gastric juice from perfectly healthy persons has a higher acidity. UMBER observed after apparent feeding with bread 3.5 p. m., and HORNBORG[4] found higher results in a boy with gastric fistula. The juice secreted before taking food contained 3.05 p. m. acid. After taking food the acidity was higher. The acidity of juice after bread was 3.65–5.11 p. m., with an average of 4.39 p. m., and the juice after meat 4.01–5.66 p. m., or an average of 4.62 p. m. There is hardly any doubt that at least a part of the hydrochloric acid of the gastric juice does not exist free in the ordinary sense, but combined with organic substances. The results obtained for the acidity of gastric juice by physical methods are nearly identical with those obtained by titration (P. FRÄNCKEL [1]).

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[1] The literature may be found in Umber's work, l. c.

[2] Bidder and Schmidt, Die Verdauungssäfte, etc., 44; Richet, l. c.; Contejean, Contributions à l'étude de la physiol. de l'estomac, Thèses, Paris, 1892.

[3] Pflüger's Arch., 90 and 103.

[4] See Richet, l. c.; Contejean, l. c.; Verhaegen, "La Cellule," 1896 and 1897; Umber, l. c.; Hornborg, l. c.; and the literature on the estimation of hydrochloric acid in the gastric contents (p. 375).

As chief organic constituent, perfectly fresh gastric juice (of dogs) contains a very complex substance (or perhaps a mixture of substances) which coagulates on boiling and which separates on strongly cooling the juice. This substance is considered by certain experimenters (NENCKI and SIEBER, and PAWLOW) as the conveyor of the several ferment actions of the gastric juice, i.e., the pepsin as well as the rennin action. Gastric juice also contains lecithin and chlorine, and yields nucleoproteid, proteose, nuclein bases, and pentose as cleavage products (NENCKI and SIEBER [2]).

The specific gravity of gastric juice is low, 1.001–1.010. It is correspondingly poor in solids. Older analyses of gastric juice from man, the dog, and the sheep were made by C. SCHMIDT.[3] As these analyses refer only to impure gastric juice they are of little value. The quantity of solids in saliva-free gastric juice from a dog was 27 p. m., with 17.1 p. m. organic substance. The quantity of free hydrochloric acid was 3.1 p. m. Besides these SCHMIDT found NaCl 1.46; $CaCl_2$ 0.6; KCl 1.1; NH_4Cl 0.5; earthy phosphates 1.9; and $FePO_4$ 0.1 p. m. NENCKI [4] found 5 milligrams sulphocyanic acid per litre of gastric juice of a dog. The pure gastric juice of another dog contained, according to NENCKI and SIEBER,[2] an average of 3.06 p. m. solids.

Besides the free hydrochloric acid, *pepsin*, *rennin*, and a *lipase* are the other physiologically important constituents of gastric juice.

Pepsin. This enzyme is found, with the exception of certain fishes, in all vertebrates thus far investigated.

Pepsin occurs in adults and in new-born infants. This condition is different in new-born animals. While in a few herbivora, such as the rabbit, pepsin occurs in the mucous coat before birth, this enzyme is entirely absent at the birth of those carnivora which have thus far been examined, such as the dog and cat.

In various invertebrates enzymes have also been found which have a proteolytic action in acid solutions. It has been shown that these enzymes, nevertheless, are not in all animals identical with ordinary pepsin. According to KLUG and WRÓBLEWSKI [5] the pepsins found in man and various higher animals are somewhat different. Enzymes also occur in various plants and animal organs; although not identical with pepsin, they act in acid reaction and stand to a certain extent between pepsin and trypsin.

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[1] Zeitschr. f. exp. Path. u. Therap., **1**.

[2] Zeitschr. f. physiol. Chem., **32**.

[3] l. c.

[4] Ber. d. d. chem. Gesellsch., **28**.

[5] Klug, Pflüger's Arch., **60**; Wróblewski, Zeitschr. f. physiol. Chem., **21**.

To this group belongs GLAESSNER's *pseudopepsin*, which according to him is the only peptic enzyme in the pyloric end. Pseudopepsin, whose existence is disputed by KLUG, while others (REACH, PEKELHARING) affirm its occurrence in the mucous membrane, acts, according to GLAESSNER, also in neutral and alkaline reaction and yields tryptophane among other cleavage products. According to BERGMANN[1] it is identical with erepsin (see below). Among the enzymes of the mucosa of the stomach belongs the so-called *antipepsin* discovered by WEINLAND,[2] which has a retarding action upon pepsin digestion and, as some claim, prevents the self-digestion of the mucous membrane.

Pepsin is as difficult to isolate in a pure condition as other enzymes. The pepsin prepared by BRÜCKE and SUNDBERG gave negative results with most reagents for proteins, and showed nevertheless a powerful action, which seems to indicate that it was very pure. SCHOUMOW-SIMANOWSKI, NENCKI and SIEBER, and also PEKELHARING have designated as the true enzyme the substance containing chlorine, which they obtained by strongly cooling the gastric juice. That this substance is not an individual, and hence cannot be pepsin, follows from the investigations of PEKELHARING. While pepsin, according to NENCKI and SIEBER, was rich in phosphorus and contained nucleoproteid, PEKELHARING's pepsin was free from phosphorus and yielded no nucleoproteid. FRIEDENTHAL and MIYAMOTA[3] have also shown that the pepsin is still active after the splitting off of the nuclein complex (and also the protein). As pepsin is readily precipitated with the proteins and combines therewith, it is difficult to decide whether pepsin is a protein substance or not, and the question as to the nature of pepsin is still undecided, just as is the case with other enzymes. As ordinarily known, pepsin, at least in an impure form, is soluble in water and glycerine. It is precipitated by alcohol, but only slowly destroyed thereby. In aqueous solution its action is quickly destroyed on heating to boiling. According to BIERNACKI[4] pepsin in neutral solutions is destroyed by heating to 55° C. In the presence of 2 p. m. HCl a temperature of 55° C. is not injurious, and the compound with acid is more resistant than the free pepsin (GROBER[5]). Pepsin in acid solution is destroyed by heating to 65° C. for five minutes. On adding peptone and certain salts the pepsin may be heated to 70° C. without decomposing. In the dry state it can, on the contrary, be heated to over 100° C. without losing its physiological action. The only property which is characteristic of pepsin is that it dissolves protein bodies in acid but not in neutral or alkaline solutions, with the formation of proteoses, peptones, and other products.

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[1] Glaessner, Hofmeister's Beiträge, 1; Klug, Pflüger's Arch., 92; Reach, Hofmeister's Beiträge, 4; Pekelharing, Arch. des scienc. biolog., St. Pétersbourg, 11; Pawlow-Festband, 1904; Bergmann, Skand. Arch. f. Physiol., 18.

[2] Zeitschr. f. Biologie, 44.

[3] Brücke, Wien. Sitzungsber., 43; Sundberg, Zeitschr. f. physiol. Chem., 9; Schoumow-Simanowski, Arch. f. exp. Path. u. Pharm., 33; Pekelharing, Zeitschr. f. physiol. Chem., 22 and 35; Nencki and Sieber, *ibid.*, 32; Friedenthal and Miyamota, Centralbl. f. Physiol., 15, 785.

[4] Zeitschr. f. Biologie, 28.

[5] Arch. f. exp. Path. u. Pharm., 51.

The methods for the preparation of relatively pure pepsin depend, as a rule, upon its property of being thrown down with finely divided precipitates of other bodies, such as calcium phosphate or cholesterin. The rather complicated methods of BRÜCKE and SUNDBERG are based upon this property. PEKELHARING makes use of a prolonged dialysis and precipitation with 0.2 p. m. HCl.

Very permanent pepsin solutions, from which the enzyme with considerable protein can be precipitated by alcohol, may be prepared by extraction with glycerine. Solutions having a strong action may also be prepared by making an infusion of the gastric mucosa of an animal in acidified water (2–5 p. m. HCl). This is unnecessary, as we can obtain pure gastric juice according to PAWLOW's method, and also because very active commercial preparations of pepsin can be bought in the market.

The Action of Pepsin on Proteins. Pepsin is inactive in neutral or alkaline reactions, but in acid liquids it dissolves coagulated protein bodies. The protein always swells and becomes transparent before it dissolves. Unboiled fibrin swells up in a solution containing 1 p. m. HCl, forming a gelatinous mass, and does not dissolve at ordinary temperature within a couple of days. Upon the addition of a little pepsin, however, this swollen mass dissolves quickly at ordinary temperatures. Hard-boiled-egg albumin, cut in thin pieces with sharp edges, is not perceptibly changed by dilute acid (2–4 p. m. HCl) at the temperature of the body in the course of several hours. But the simultaneous presence of pepsin causes the edges to become clear and transparent, blunt and swollen, and the protein gradually dissolves.

From what has been said above in regard to pepsin, it follows that proteins may be employed as a means of detecting pepsin in liquids. Ox-fibrin may be employed as well as coagulated egg-albumin, which latter is used in the form of slices with sharp edges. As the fibrin is easily digested at the normal temperature, while the pepsin test with egg-albumin requires the temperature of the body, and as the test with fibrin is somewhat more delicate, it is often preferred to that with egg-albumin. When we speak of the "*pepsin test*" without further explanation, we ordinarily understand it as the test with fibrin.

This test, nevertheless, requires care. The fibrin used should be ox-fibrin and not pig-fibrin, which last is dissolved too readily with dilute acid alone. The unboiled ox-fibrin may be dissolved by acid alone without pepsin, but this generally requires more time. In testing with unboiled fibrin at normal temperature, it is advisable to make a control test with another portion of the same fibrin with acid alone. Since at the temperature of the body unboiled fibrin is more easily dissolved by acid alone, it is best always to work with boiled fibrin.

As pepsin has not thus far been prepared in a positively pure condition, it is impossible to determine the absolute quantity of pepsin in a liquid. It is only possible to compare the relative amounts of pepsin in two or more liquids, which may be done in several ways.

The older method, that of **Brücke**, consists in diluting the two pepsin solutions to be compared with certain proportions of 1 p. m. hydrochloric acid, so that when the amount of pepsin contained in the original solution is equal to 1, each solution contains a degree of dilution, p, corresponding to 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, etc. A flock of fibrin or a piece of hard-boiled egg is added to each test and the time noted when each test begins to digest and when it ends. The relative amount of pepsin is calculated from the rapidity of digestion as follows: the tests $p=\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, of one series is digested in the same time as tests $p=1$, $\frac{1}{2}$, $\frac{1}{4}$ of the other series; hence the first solution contained four times as much pepsin. This method is not used as often as the following:

Mett's Method. Draw up white of egg in a glass tube 1–2 millimetres in diameter, coagulate it by plunging it into hot water at 95° C., and cut the ends off sharply; then add two tubes to each test-tube with a few cubic centimetres of the acid pepsin solution; allow them to digest at body temperature, and after a certain time, generally after ten hours, measure the lineal extent of the digested layer of albumin in the various tests, bearing in mind that the digested layer at each end must not be longer than 6–7 millimetres. The quantity of pepsin in the comparative tests is as the square of the millimetres of the albumin-column dissolved in the same time. Thus if in one case 2 millimetres of albumin were dissolved and in the other 3 millimetres, then the quantity of pepsin is as 4:9. If the fluid removed from the stomach, which is rich in bodies having a disturbing influence upon pepsin digestion, is to be tested, then the liquid must be first properly diluted with 2–4 p. m. hydrochloric acid (**Nierenstein** and **Schiff**[1]).

Objections have been raised against these methods from several sides, especially by **Grützner**, but they can be recommended for practical purposes as being simple and rather accurate. **Huppert** and E. **Schütz** measure the relative quantities of pepsin from the amount of secondary proteoses formed under certain conditions. The proteoses were determined by the polariscope. J. **Schütz** determines the total proteose-nitrogen, and **Spriggs**[2] finds that the change in the viscosity is a measure of the amount of pepsin.

Volhard and **Löhlein**[3] use an acid casein solution for the pepsin determination, and determine, after precipitation with sodium sulphate, the acidity of the filtrate of the digested test as well as of the original control solution. The casein is precipitated as an acid compound by the sulphate, and the filtrate separated from the precipitate contains less acid than the original solution. In proportion as the digestion progresses less substance is precipitated by the sulphate, and the acidity of the filtrate becomes correspondingly higher. The increase in acidity in the different portions varies within certain limits as the square root of the quantity of ferment.

Grützner's[4] test is based on the use of finely cut fibrin colored with carmine. The fibrin is first allowed to swell up in 1 p. m. hydrochloric acid and then about equal quantities are placed in test-tubes of the same diameter and treated with 15 c.c. of 1 p. m. hydrochloric acid. After the addition of the pepsin solution to be tested the fibrin dissolves, giving a red color to the solution, and the strength of the digestion is determined by the depth of the color. For comparison a series of tubes are used containing carmine solution diluted in known proportions, and which are arranged so that, for example, when one pepsin solution had a color corresponding to No. 1, and the other, on the contrary, to No. 4, then the latter had four times as much fibrin dissolved as the first.

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[1] Mett, see Pawlow, l. c., 31; Nierenstein and Schiff, Berl. klin. Wochenschr., 40.

[2] Huppert and Schütz, Pflüger's Arch., 80; J. Schütz, Zeitschr. f. physiol. Chem., 30; Spriggs, *ibid.*, 35.

[3] Hofmeister's Beiträge, 7.

[4] Grützner, Pflüger's Arch., 8 and 106. See also A. Korn, "Über Methoden P quantitativ zu bestimmen," Inaug.-Dissert., Tübingen, 1902.

The *rapidity of the pepsin digestion* depends on several circumstances. Thus *different acids* are unequal in their action; hydrochloric acid shows in slight concentration, 0.8–1.8 p. m., a more powerful action than any other acid, whether inorganic or organic. In greater concentration other acids may have a powerful action; but no constant relationship has been found between the strength of various acids and their action in pepsin digestion, and the statements in regard to the action of different acids are somewhat contradictory.[1] Sulphuric acid, it seems, has a weaker action than the other inorganic acids. The *degree of acidity* is also of the greatest importance. With hydrochloric acid the degree of acidity is not the same for different protein bodies. For fibrin it is 0.8–1 p. m., for myosin, casein, and vegetable proteins about 1 p. m., for coagulated egg-albumin, on the contrary, about 2.5 p. m. The rapidity of the digestion increases, at least to a certain point, with the *quantity of pepsin* present, unless the pepsin added is contaminated by a large quantity of the products of digestion, which may prevent its action.

According to E. SCHÜTZ,[2] whose statements have been confirmed by several others, the digestion products produced in a certain time are, within certain limits, proportional to the square root of the relative amounts of pepsin (the SCHÜTZ-BORISSOW rule). The *accumulation of products of digestion* has a retarding action on digestion, although, according to CHITTENDEN and AMERMAN,[3] the removal of the digestion products by means of dialysis does not essentially change the relationship between the proteoses and true peptones. Pepsin acts more slowly at low temperatures than it does at higher ones. It is even active in the neighborhood of 0° C., but digestion takes place very slowly at this temperature. With increasing temperature the rapidity of digestion also increases until about 40° C., when the maximum is reached. According to the investigations of FLAUM[4] it is probable that the relationship between proteoses and peptones remains the same, irrespective of whether the digestion takes place at a low or high temperature, as long as the digestion is continued for a long enough time.

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[1] See Wróblewski, Zeitschr. f. physiol. Chem., **21**, and especially Pfleiderer, Pflüger's Arch., **66**, which also gives references to other works; Larin, Biochem. Centralbl., **1**, 484; and A. Pick, Wien. Sitzungsber., M. N. Klasse, **112**.

[2] Zeitschr. f. physiol. Chem., **9**.

[3] Journ. of Physiol., **14**.

[4] Zeitschr. f. Biologie, **28**.

If the *swelling up of the protein* is prevented, as by the addition of neutral salts, such as NaCl, in sufficient amounts, or by the addition of bile to the acid liquid, digestion can be prevented to a greater or less extent. *Foreign bodies* of different kinds produce different actions, in which naturally the variable quantities in which they are added are of the greatest importance. Salicylic acid and carbolic acid, and especially sulphates (Pfleiderer), retard digestion, while arsenious acid promotes it (Chittenden), and hydrocyanic acid is relatively indifferent. By experiments with salt solutions so strongly diluted that the action, on account of the strong dissociation, was brought about by ions and not by the electrolytically neutral molecules (min. $\frac{1}{40}$ and max. $\frac{1}{4}$ normal salt solutions), J. Schütz[1] found that the anions had a much greater retarding action upon pepsin digestion than the cations. Of these latter the sodium cation had the strongest retarding action. Alcohol in large quantities (10 per cent and above) disturbs the digestion, while small quantities act indifferently. Metallic salts in very small quantities may indeed sometimes accelerate digestion, but otherwise they tend to retard it. The action of metallic salts in different cases can be explained in various ways, but they often seem to form with proteins insoluble or difficultly soluble combinations. The alkaloids may also retard the pepsin digestion (Chittenden and Allen[2]). A very large number of observations have been made in regard to the action of foreign substances on artificial pepsin digestion, but as these observations have not given any direct result in regard to the action of these same substances on natural digestion, as well as upon secretion and absorption, we will not discuss them here.

The Products of the Digestion of Proteins by Means of Pepsin and Acid. In the digestion of nucleoproteids or nucleoalbumins an insoluble residue of nuclein or pseudonuclein always remains, although under certain circumstances a complete solution may occur. Fibrin also yields an insoluble residue, which consists, at least in great part, of nuclein, derived from the form-elements enclosed in the blood-clot. This residue which remains after the digestion of certain proteins was called *dyspeptone* by Meissner. This name is therefore not only unnecessary but indeed erroneous, as this residue does not consist of bodies related to the peptones. In the digestion of proteins, substances similar to acid albuminates, *parapeptone* (Meissner[3]), *antialbumate*, and *antialbumid* (Kühne), may also be formed. On separating these bodies the filtered liquid, neutralized at boiling-point, contains *proteoses* and *peptones* in the old sense, while the so-called KÜHNE true peptone and the other cleavage products are obtained only after a longer and more intense digestion. The relationship between the various proteoses changes very much in different cases and in the digestion of the various proteins. For instance, a larger quantity of primary proteoses is obtained from fibrin than from hard-boiled-egg albumin or from the proteins of meat; and the different proteins, according to the researches of KLUG,[1] yield on pepsin digestion unequal quantities of the various digestive products. In the digestion of unboiled fibrin an intermediate product may be obtained in the earlier stages of the digestion—a globulin which coagulates at 55° C. (HASEBROEK [2]). For information in regard to the different proteoses and peptones which are formed in pepsin digestion the reader is referred to previous pages (50 to 61).

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[1] Hofmeister's Beiträge, 5.

[2] Studies from the Lab. Physiol. Chem. Yale University, 1, 76. See also Chittenden and Stewart, *ibid.*, 3, 60.

[3] The works of Meissner on pepsin digestion are found in Zeitschr. f. rat. Med., 7, 8, 10, 12, and 14.

Action of Pepsin-Hydrochloric Acid on Other Bodies. The *gelatine-forming substance* of the connective tissue, of the cartilage, and of the bones, from which last the acid dissolves only the inorganic substances, is converted into *gelatine* by digesting with gastric juice. The gelatine is further changed so that it loses its property of gelatinizing and is converted into gelatoses and peptone (see page 79). True *mucin* (from the submaxillary) is dissolved by the gastric juice, yielding substances similar to peptone and a reducing substance similar to that obtained by boiling with a mineral acid. *Mucoids* from tendons, cartilage, and bones dissolve, according to POSNER and GIES,[3] in pepsin-hydrochloric acid, but leave a residue which amounts to about 10 per cent of the original material and which, as it seems, consists in great part, if not entirely, of a combination of proteid with glucothionic acid (Chapters VII and VIII). The solution contains primary and secondary mucoproteoses and mucopeptones. The former contain glucothionic acid, but the latter do not. *Elastin* is dissolved more slowly and yields the above-mentioned substances (page 76). *Keratin* and the epidermal formations are insoluble. The *nucleins* are dissolved with difficulty, and the cell nuclei, therefore, remain in great part undissolved in the gastric juice. The *animal cell-membrane* is, as a rule, more easily dissolved the nearer it stands to elastin, and it dissolves with greater difficulty the more closely it is related to keratin. The *membrane of the plant-cell* is not dissolved. *Oxyhæmoglobin* is changed into hæmatin and protein, the latter undergoing further digestion. It is for this reason that blood is changed into a dark-brown mass in the stomach. The gastric juice does not act upon *fat*, but, on the contrary, dissolves the cell-membrane of fatty tissue, setting the fat free. Gastric juice has no action on starch or the simple varieties of sugar. The statements in regard to the ability of gastric juice to invert cane-sugar are very contradictory. At least this action of the gastric juice is not constant, and if it is present at all it is probably due to the action of the acid.

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[1] Pflüger's Arch., **65**.

[2] Zeitschr. f. physiol. Chem., **11**.

[3] Amer. Journ. of Physiol., **11**.

Pepsin alone, as above stated, has no action on proteins, and an acid of the intensity of the gastric juice can only very slowly, if at all, dissolve coagulated albumin at the temperature of the body. Pepsin and acid together not only act more quickly, but qualitatively they act otherwise than the acid alone, at least upon dissolved protein. This has led to the assumption of the presence of a *pepsin-hydrochloric acid* whose existence and action are only hypothetical. As pepsin digestion, it seems, yields finally the same products as the hydrolytic cleavage with acids, we can say for the present only that this enzyme acts like other catalyzers in very powerfully accelerating a process which would proceed also without the catalyzers.

Chymosin (RENNIN) and **Parachymosin.** So far two different rennet enzymes have been obtained from animal stomachs, namely, the enzyme called rennin (chymosin), which is found in the calf's stomach and has been known for a long time, and the parachymosin discovered by BANG[1] which is the rennet enzyme of the human stomach. This latter occurs in the gastric juice of man under physiological conditions, but may be absent under special pathological conditions (SCHUMBURG, BOAS, JOHNSON, KLEMPERER[2]). Chymosin is habitually found in the neutral, watery infusion of the fourth stomach of the calf and sheep, especially in an infusion of the fundus part. In other mammals and in birds it is seldom found, and in fishes hardly ever in the neutral infusion. In these cases, as in man and the higher animals, a rennin-forming substance, a *rennin zymogen*, occurs, which is converted into rennin by the action of an acid (HAMMARSTEN). We have no knowledge as to whether the rennet enzyme found in different animals is chymosin or parachymosin. Enzymes acting like rennin are also found in the blood and several organs of higher animals, as well as in invertebrates. Similar enzymes also occur widely diffused in the plant kingdom, and numerous micro-organisms have the power of producing rennin enzymes. Also antibodies to the rennet enzymes, *antichymosins*, occur in the animal kingdom, as shown by HAMMARSTEN and RÖDÉN in blood-serum, and may be produced in the animal body by the introduction of rennin into the latter (MORGENROTH[3]).

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[1] Deutsch. med. Wochenschr , 1899, and Pflüger's Arch., 79.

[2] Schumburg, Virchow's Arch., 97. A good review of the literature may be found in Szydlowski, Beiträge zur Kenntnis des Labenzym nach Beobachtungen an Säuglingen, Jahrb. f. Kinderheilkunde (N. F.), 34. See also Lörcher, Pflüger's Arch., 69, which also contains the pertinent literature. An excellent review of the literature on rennin and its action may be found in E. Fuld, Ergebnisse der Physiol , 1, Abt. 1, 468.

[3] See Rödén, Upsala Läkaref. Förh., 22; Morgenroth, Centralbl. f. Bakter., 26 and 27.

Rennin is just as difficult to prepare in a pure state as the other enzymes. The purest rennin enzyme thus far obtained did not give the ordinary protein reactions. On heating its solution rennin is more or less quickly destroyed, depending upon the length of heating and upon the concentration. If an active and strong infusion of the gastric mucosa in water containing 3 p. m. HCl is heated to 37–40° C. for 48 hours, the rennin is destroyed, while the pepsin remains. A pepsin solution free from rennin can be obtained in this way. Rennin is characterized by its physiological action, which consists in coagulating milk or a casein solution containing lime, if neutral or very faintly alkaline. The law of the action of this enzyme is different from that of the action of pepsin. As specially shown by FULD, within certain limits, the coagulation time, T, is equal to a constant, C, divided by the quantity of rennin, L. As shown by BANG,[1] this law does not apply to parachymosin.

From the different laws of pepsin and rennin action it follows that the repeated appearance recently of the view of PAWLOW'S school, that pepsin and rennin are the same bodies, cannot be correct. The experiments given by PAWLOW and PARASTSCHUK as proof for this view are unfortunately in error in principle. Also the investigations published by SAWJALOW are not decisive, and the recent work of SCHMIDT-NIELSEN [2] has given new proofs for the non-identity of the two enzymes. According to NENCKI and SIEBER, with whom PEKELHARING agrees, the enzyme of the gastric juice forms a gigantic molecule which is able to perform the different actions at the same time, although each enzyme action is connected with a certain atomic complex. Such a view might appear plausible, especially as pepsin and rennin enzyme regularly occur together in the animal kingdom. As the body which is precipitated from gastric juice by cooling and which forms the ferment has been shown to be a mixture, and also as the two enzymes and also the proenzymes have been separated by GLAESSNER [3] from each other, this view does not seem to be sufficiently well grounded.

Parachymosin differs from chymosin by being much more resistant towards acids, but is more readily destroyed by alkalies. Calcium chloride accelerates the casein coagulation with parachymosin very much more than with chymosin.

Rennin, like other enzymes, may be carried down by other precipitates and thus may be obtained relatively pure. It may also be obtained, contaminated with a great deal of proteins, by extracting the mucous coat of the stomach with glycerine.

A comparatively pure solution of rennin may be obtained in the following way. An infusion of the mucous coat of the stomach in hydrochloric acid is prepared and then neutralized, after which it is repeatedly shaken with new quantities of magnesium carbonate until the pepsin is precipitated. The filtrate, which should act strongly on milk, is precipitated by basic lead acetate, the precipitate decomposed with very dilute sulphuric acid, the acid liquid filtered and treated with a solution of stearin soap. The rennin is carried down by the fatty acids set free, and when these last are placed in water and removed by shaking with ether, the rennin remains in the watery solution.

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[1] Fuld, Hofmeister's Beiträge, **2**; Bang, l. c.

[2] Pawlow and Parastschuk, Zeitschr. f. physiol. Chem., **42**; Sawjalow, *ibid.*, **46**; Schmidt-Nielsen, *ibid.*, **48**.

[3] Glaessner, Hofmeister's Beiträge, **1**; Nencki and Sieber, Zeitschr. f. physiol. Chem., **32**; Pekelharing, foot-notes, and 3, p. 355.

Plastein. As mentioned on page 56, DANILEWSKY first showed the power of rennin solutions of causing a partial coagulation of proteoses and of converting them into so-called plastein. This action, which is also ascribed to other enzyme solutions (see page 56), has probably nothing to do with the rennin enzyme, but depends more likely upon another enzyme. The nature of these enzymes, as well as the manner and importance of the plastein formation, is still unknown.

Gastric Lipase (STOMACH STEAPSIN). F. VOLHARD[1] has made the discovery that the gastric juice has a strong fat-splitting action only when the fat is in a fine emulsion, as in the yolk of the egg, in milk or in cream. This action, which is still not undisputed (INOUYE), depends upon an enzyme which can be extracted from the mucosa by glycerine, and whose action, it seems, follows SCHÜTZ's law for pepsin, that the quantity is proportional to the square of the quantity of enzymotic products. This enzyme, which seems to be produced from a zymogen, does not behave in the same way when obtained from different animals. The enzyme from the pig stomach is very sensitive towards acids but less sensitive towards alkalies. That from the dog and from the human stomach is, on the contrary, sensitive towards alkalies, while comparatively resistant towards acids (FROMME). The enzyme is only slowly extracted from the pig stomach by glycerine, and the extract shows its maximal activity only after several days. The filtered glycerine extract is inactive (FROMME). FALLOISE, by experiments on rabbits and dogs, has shown that gastric lipase is not derived by regurgitation from the intestine, nor does it come from the pancreas by means of the blood, but it is formed in the stomach. LAQUEUR, by means of experiments with the filtered juice obtained from a small PAWLOW stomach, has shown that this juice had a splitting action upon yolk emulsion. Gastric lipase, according to LAQUEUR,[2] cannot be pancreas steapsin, and is also not an intracellular tissue enzyme, but is secreted by the glands.

The question whether the parietal cells principally or the chief cells, or both, take part in the formation of free acid is somewhat disputed.[3]

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[1] Volhard, Münch. med. Wochenschr., 1900, and Zeitschr. f. klin. Med., **42**, **43**. See also Stade, Hofmeister's Beiträge, **3**; A. Fromme, *ibid.*, **7**; A. Zinsser, *ibid.*; H. Engel, *ibid.*; and Inouye, Arch. f. Verdauungskrank., **9**.

[2] Falloise, Arch. int. de Physiol., **3** (1906); Laqueur, Hofmeister's Beiträge, **8**.

[3] See Heidenhain, Pflüger's Arch., **18** and **19**, and Hermann's Handbuch, **5**, part I, "Absonderungsvorgänge"; Klemensiewicz, Wien. Sitzungsber., **71**; Fränkel, Pflüger's

There can be no doubt that the hydrochloric acid of the gastric juice originates from the chlorides of the blood, because, as is well known, a secretion of perfectly typical gastric juice takes place in the stomachs of fasting or starving animals. As the chlorides of the blood are derived from the food, it is easily understood, as shown by CAHN,[1] that in dogs after a sufficiently long common-salt starvation the stomach secreted a gastric juice containing pepsin, but no free hydrochloric acid. On the administration of soluble chlorides, a gastric juice containing hydrochloric acid was immediately secreted. On the introduction of alkali iodides or bromides, KÜLZ, NENCKI and SCHOUMOW-SIMANOWSKI[2] have shown that the hydrochloric acid of the gastric juice is replaced by HBr, and to a less extent by HI. According to KOEPPE the seat of formation of hydrochloric acid is not the blood nor the glands, but the interior of the stomach in the immediate neighborhood of the wall. The hydrochloric acid is assumed to be produced from the chlorides of the food, as the semipermeable wall is not permeable for the Cl ions but is for the Na ions and for the H ions. As the Na ions leave the stomach contents an equivalent quantity of H ions wander from the blood through the stomach wall into the interior of the stomach and combine with the Cl ions. This theory is difficult to reconcile with the fact that in dogs with apparent feeding the empty stomach secretes abundant juice. Other objections have also been raised against this by BENRATH and SACHS. The secretion of free hydrochloric acid from the alkaline blood has been explained in other ways (MALY, BUNGE, L. SCHWARZ), but as yet no satisfactory theory has been suggested.[3]

After a full meal, when the store of pepsin in the stomach is completely exhausted, SCHIFF claims that certain bodies, especially dextrin, have the property of causing a supply of pepsin in the mucous membrane. This "charge theory," though experimentally tested by several investigators, has nevertheless not yet been confirmed. On the contrary, the statement of SCHIFF that a substance forming pepsin, a "*pepsinogen*" or "*propepsin*," occurs in the ventricle has been proved. LANGLEY[4] has shown positively the existence of such a substance in the mucous coat. This

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Arch., **48** and **50**; Contejean, l. c., Chapter II; Kranenburg, Archives Teyler, Series II, Haarlem, 1901, and Mosse, Centralbl. f. Physiol., **17**, 217.

[1] Zeitschr. f. physiol. Chem., **10**.

[2] Külz, Zeitschr. f. Biologie, **23**; Nencki and Schoumow, Arch. des sciences biol. de St. Pétersbourg, **3**.

[3] Koeppe, Pflüger's Arch., **62**; Benrath and Sachs, *ibid.*, **109**; Maly, see v. Bunge's Lehrbuch der physiol. u. pathol. Chem., 4. Aufl., 1898; Schwarz, Hofmeister's Beiträge, **5**.

[4] Schiff, Leçons sur la physiol. de la digéstion, 1867, **2**; Langley and Edkins, Journ. of Physiol., **7**.

substance, propepsin, shows a comparatively strong resistance to dilute alkalies (a soda solution of 5 p. m.), which easily destroy pepsin (LANGLEY). Pepsin, on the other hand, withstands better than propepsin the action of carbon dioxide, which quickly destroys the latter. The occurrence of a rennin zymogen, and possibly also of a steapsinogen, in the mucous coat has been mentioned above.

According to HERZEN and his collaborators[1] we must differentiate between pepsinogens and bodies accelerating the flow of juice. To the first belong inulin and glycogen, while alcohol belongs to the latter class of bodies. Dextrin not only accelerates the flow of juice, but also acts as a pepsinogen, especially as the latter. Meat extract which has both actions is especially a flow-accelerator. The pepsinogen action consists in converting the zymogen into pepsin and in this way increases the quantity of pepsin; the flow-accelerating substances increase the quantity of secreted juice.

The question in what cells the two zymogens, especially the propepsin, are produced has been extensively discussed for several years. Formerly it was the general opinion that the parietal cells were pepsin cells, but since the investigations of HEIDENHAIN and his pupils, LANGLEY and others, the formation of pepsin has been attributed to the chief cells.[2]

The Pyloric Secretion. That part of the pyloric end of the dog's stomach which contains no fundus glands was dissected by KLEMENSIEWICZ, one end being sewed together in the shape of a blind sac and the other sewed into the stomach. From the fistula thus created he was able to obtain the pyloric secretion of a living animal. This secretion is alkaline, viscous, jelly-like, rich in mucin, of a specific gravity of 1.009–1.010, and containing 16.5–20.5 p. m. solids. It habitually contains pepsin, which has been proved by HEIDENHAIN by observations on a permanent pyloric fistula, and the amount may sometimes be considerable. CONTEJEAN has investigated the pyloric secretion in other ways, and finds that it contains both acid and pepsin. The alkaline reaction of the secretions investigated by HEIDENHAIN and KLEMENSIEWICZ is due, according to CONTEJEAN, to an abnormal secretion caused by the operation, because the stomach readily yields an alkaline juice instead of an acid one under abnormal conditions. The statements of HEIDENHAIN and KLEMENSIEWICZ have been substantiated by AKERMANN, while KRESTEFF, who operated according to another method, and SCHEMIAKINE[3] have come to the same results. KRESTEFF found in the juice (of dogs) pepsin, but no chymosin. VERHAEGEN[4] has observed in human beings towards the end of the ventricle digestion a non-fluid acid, which, according to him, originates in the pyloric region.

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[1] Pflüger's Arch., 84.

[2] See foot-note 1, p. 364.

[3] Heidenhain and Klemensiewicz, l. c.; Contejean, l. c., Chapter II, and Skand. Arch. f. Physiol., 6; Akermann, *ibid.*, 5; Kresteff, Maly's Jahresber., **30**; Schemiakine, Arch. des scienc. biolog. de St. Pétersbourg, **10**.

[4] See the work of Verhaegen, "La Cellule," 1896, 1897.

The secretion of gastric juice under different conditions may vary considerably. The statements concerning the quantity of gastric juice secreted in a certain time are therefore so unreliable that they need not be taken into account.

The Chyme and the Digestion in the Stomach. By means of the chemical stimulation caused by the food, a copious secretion of gastric juice occurs, which gradually mixes with the swallowed food, and digests it more or less strongly. The material in the stomach during digestion which has a pasty or thick consistency and is called chyme, is not a homogeneous mixture of the ingesta with the various digestive fluids, gastric juice, saliva, and gastric mucus, but the conditions seem to be more complicated.

From the investigations of several workers, such as HOFMEISTER and SCHÜTZ, MORITZ, CANNON, SCHEMIAKINE,[1] and others, on the movements of the stomach, we conclude that this organ consists of two physiologically different parts, the pylorus and the fundus. The greater fundus part, which serves essentially as a reservoir, may by a rhythmic, strong contraction of the muscle, acting like a sphincter between it and the pylorus part, be separated from the latter, and according to some observers so completely so that during contraction almost nothing passes from the fundus to the pylorus part. Differing from the fundus part, the pylorus is the seat of very powerful contractions by which its contents are intimately mixed with gastric juice and are also driven through the pyloric valve into the intestine.

The contents of the pylorus part have an acid reaction, and a strong pepsin digestion takes place in the contents, which are thoroughly mixed with gastric juice. By very instructive investigations on different animals (frogs, rats, rabbits, guinea-pigs, and dogs) GRÜTZNER[2] has shown, when the animals are fed with food having different colors, and the stomach removed after a certain time, and the contents frozen, that the frozen sections show a regular stratification of the contents. These layers are so arranged that the food first taken is found in direct contact with the mucosa, while the food taken later is inclosed by that partaken of first, and this prevents contact with the walls of the stomach. The empty stomach, whose walls touch each other, is so filled that, as a rule, the foodstuffs taken later are in the middle of the older food.

Because of this fact only the foodstuffs which lie close to the surface of the mucous membrane undergo the process of peptic digestion, and it is principally this ingesta, which lies on the surface and is laden with pepsin and mixed with gastric juice, which is shoved to the pylorus end, here mixed and digested, and finally moved into the intestine. The fundus part is therefore less a digestion-organ than a storage-organ, and in the interior of the same the food may remain for hours without coming in contact with a trace of gastric juice.

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[1] Hofmeister and Schütz, Arch. f. exp. Path. u. Pharm., 20; Moritz, Zeitschr. f. Biologie, 32; Cannon, Amer. Journ. of Physiol., 1; Schemiakine, l. c.

[2] Pflüger's Arch., 106.

What has been said above applies at least to solid food. We have no extensive observations on the behavior of fluids or semifluid food. According to GRÜTZNER, also in these cases, as well as in the above-mentioned experiments, the swallowed foodstuffs are not irregularly mixed together. Fluids quickly leave the stomach, which is also the case with a mixture of solid and fluid food.

The fact that only that part of the ingesta lying on the mucous membrane is mixed with gastric juice, while the mass in the interior is not acid in reaction, is of special importance for the digestion of starches in the stomach. By this we can explain why the salivary diastase, although sensitive towards acids, can continue its action for a long time in the contents of the stomach. CANNON and DAY[1] have shown that this is true by special experiments upon animals, and the occurrence of sugar and dextrin in the contents of the human stomach has been repeatedly observed. In carnivora, whose saliva shows nearly no diastatic action, it is *a priori* not expected that there should be a diastatic action in the stomach—of course excluding the action of micro-organisms which may be present. Still, according to FRIEDENTHAL, dogs can readily digest starch, and the gastric juice of dogs, according to him, contains a diastatic enzyme which is active even in strong acid reaction.[2]

The gastric contents which have been prepared in the pylorus part are passed through the pylorus into the intestine intermittently. This material is generally fluid, but it is possible that pieces of solid food may also occur, and this has been often observed. From BUSCH's observations on a human intestinal fistula, it required generally 15–30 minutes after eating for undigested food to pass into the upper part of the small intestine. In a case of duodenal fistula in a human being observed by KÜHNE, he saw, ten minutes after eating, uncurdled but still coagulable milk and small pieces of meat pass out of the fistula. The time in which the stomach unburdens itself of its contents depends naturally upon the coarseness or fineness of the food; essentially, however, it depends upon the reflex action from the stomach or intestine causing an opening or closing of the pylorus, which action is dependent upon the quantity and character of the food, the amount of fat, and the degree of acidity in the contents of the stomach and intestine. The emptying of the food into the small intestine causes, as shown by PAWLOW, a closing of the pylorus by chemoreflex in which the hydrochloric acid and the fat take part, and we thus find in this regard an alternate action between the stomach and duodenum. The time necessary for the stomach to empty itself must differ considerably under various conditions. BEAUMONT[1] found in his extensive observations on the Canadian hunter ST. MARTIN that the stomach, as a rule, is emptied 1½–5½ hours after a meal, depending upon the character of the food.

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[1] Cannon and Day, Amer. Journ. of Physiol., 9; Friedenthal, Arch. f. (Anat. u.) Physiol., 1899, Suppl.

[2] See Scheunert and Grimmer, Zeitschr. f. physiol. Chem., 48, on the occurrence of diastatic enzymes in plant foodstuffs.

The time in which different foods leave the stomach depends also upon their digestibility. In regard to the unequal digestibility in the stomach of foods rich in proteins, which really form the object of the action of the gastric juice, a distinction must be made between the rapidity with which the proteins are converted into proteoses and peptones and the rapidity with which the food is converted into chyme, or at least so prepared that it may easily pass into the intestine. This distinction is especially important from a practical standpoint. When a proper food is to be decided upon in cases of diminished gastric digestion, it is important to select such foods as leave the stomach easily and quickly, independently of the difficulty or ease with which their protein is peptonized, and which require as little action as possible on the part of this organ. From this point of view those foods, as a rule, are most digestible which are fluid from the start or may be easily liquefied in the stomach; but these foods are not always the most digestible in the sense that their protein is most easily peptonized. As an example, hard-boiled white of egg is more easily peptonized than fluid white of egg at a degree of acidity of 1–2 p. m. HCl;[2] nevertheless we consider, and justly, that an unboiled or soft-boiled egg is easier to digest than a hard-boiled one. Likewise uncooked meat, when it is not chopped very fine, is not more quickly but more slowly peptonized by the gastric juice than the cooked, but if it is divided sufficiently fine it is often more quickly peptonized than the cooked.

The greater or less facility with which the different protein foods are digested in the stomach has been comparatively little studied. The most complete investigations on this subject are those of FERMI,[3] but as they do not allow of a short discussion we must refer to the original publication.

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[1] Busch, Virchow's Arch., **14**; Kühne, Lehrb. d. physiol. Chem., 53; (Pawlow and) Serdjukow, Maly's Jahresber., **29**; Lintwarew, Biochem. Centralbl., **1**, 96. See also Richet, l. c.; Beaumont, The Physiology of Digestion, 1833.

[2] Wawrinsky, Maly's Jahresber., **3**.

[3] Arch. f. (Anat. u.) Physiol., 1901, Suppl.

The rapidity with which different foods leave the stomach has been studied by CANNON[1] in the case of cats. After preliminary hunger the animals received different food, such as meat, fat, and carbohydrate mixed with bismuth subnitrate, and then with the aid of the RÖNTGEN rays the time was noted when the food passed into the intestine. The carbohydrate leaves the stomach first, the proteins next, and the fats last. If the carbohydrate is given before the protein food, then it leaves the stomach with ordinary rapidity; while if protein food and then carbohydrate is given the passage of the carbohydrate is retarded. A mixture of protein food and carbohydrates leaves the stomach more slowly than carbohydrates alone, but faster than protein food alone. The fat, which remains in the stomach for a long time and leaves the stomach only in amounts which are absorbed or removed from the duodenum, retards the passage of the protein foods as well as the carbohydrates.

As our knowledge of the digestibility of the different foods in the stomach is slight and dubious, so also our knowledge of the action of other bodies, such as alcoholic drinks, bitter principles, spices, etc., on the natural digestion is very uncertain and imperfect. The difficulties which stand in the way of this kind of investigation are very great, and therefore the results obtained thus far are often ambiguous or conflict with each other. For example, certain investigators have observed that small quantities of alcohol or alcoholic drinks do not prevent but rather facilitate digestion; others observe only a disturbing action, while other investigators report having found that the alcohol first acts somewhat as a disturbing agent, but afterwards, when it is absorbed, produces an abundant secretion of gastric juice, and thereby facilitates digestion (GLUZINSKI, CHITTENDEN[2]). The accelerating action of alcohol upon the flow of gastric juice has already been mentioned on page 352.

The digestion of sundry foods is not dependent on one organ alone, but is divided among several. For this reason it is to be expected that the various digestive organs can act for one another to a certain point, and that therefore the work of the stomach could be taken up more or less by the intestine. This in fact is the case. Thus the stomachs of dogs and cats have been completely extirpated or nearly so (CZERNY, CARVALLO and PACHON), and also that part necessary in the digestive process has been eliminated by plugging the pyloric opening (LUDWIG and OGATA), and in both cases it was possible to keep the animal alive, well fed, and strong for a shorter or longer time. This is also true for human beings.[3]

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[1] Amer. Journ. of Physiol., 12.

[2] Gluzinski, Deutsch. Arch. f. klin. Med., 39; Chittenden, Centralbl. f. d. med. Wissensch., 1889; Chittenden and Mendel and others, Amer. Journ. of Physiol., 1.

[3] Czerny, cited from Bunge, Lehrbuch d. physiol. u. path. Chem. 4. Aufl., Theil 2, 173; Carvallo and Pachon, Arch. d. Physiol. (5), 7; Ogata, Arch. f. (Anat. u.)

In these cases it is evident that the digestive work of the stomach was taken up by the intestine; but all food cannot be digested in these cases to the same extent, and the connective tissue of meat especially is sometimes found to a considerable extent undigested in the excrements.

In order to judge of the rôle of the stomach in digestion the amount of the digestion products in the stomach has been determined. These determinations, partly on man and partly on animals, have led, as is to be expected, to varying results (CAHN, ELLENBERGER and HOFMEISTER, CHITTENDEN and AMERMAN). The recent investigations of E. ZUNZ show that boiled meat in the stomach of a dog yields chiefly proteoses with small amounts of simpler cleavage products, and only very little acid albumin is formed. The quantity of proteose nitrogen was 90 per cent of the nitrogen of the non-coagulable substances. TOBLER, who has studied in dogs with duodenal fistula, the digestion in the stomach under conditions which were as nearly physiologically normal as possible has arrived at different results. After feeding finely chopped meat freed from extractives by water, he found that about 20 to 30 per cent of the total nitrogen was absorbed in the stomach, while about 20 per cent left the stomach in an insoluble form and 50 to 65 per cent in solution. The chief mass of the dissolved protein (about 80 per cent) consisted of peptones, the remainder of proteoses. LONDON and SULIMA[1] have arrived at different results. They experimented upon dogs, making various fistulæ, such as gastric, pyloric, and duodenal fistulæ, and used as food partly hard-boiled egg, which was given to the dogs in quantities of 200 grams and in as large pieces as possible, and partly raw white of egg. In both cases, contrary to the experience of TOBLER, the proteins were not absorbed in the stomach. They also found in the dog with pyloric fistula that after feeding 200 grams boiled protein the chief portion of the digestion products (about 87 per cent) left the stomach within the first two hours of digestion. Of the cleavage products the contents of the stomach contained chiefly proteoses, while in the material which left the stomach in the dog with pyloric fistula, the peptones prevailed. The proteoses seem to be retained for a longer time in the stomach, making a further transformation into peptones possible.

As is to be expected, the behavior is not always the same, and important variations often occur. It is, however, quite generally assumed that no peptonization of the proteins worth mentioning occurs in the stomach,

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Physiol., 1883; Grohé, Arch. f. exp. Path. u. Pharm., **49**. In regard to a human case of Schlatter see Wróblewski, Centralbl. f. Physiol., **11**, 665.

[1] Cahn, Zeitschr. f. klin. Med., **12**; Ellenberger and Hofmeister, Arch. f. (Anat. u.) Phyisol., 1890; Chittenden and Amerman, Journ. of Physiol., **14**; E. Zunz, Hofmeister's Beiträge, **3**. See Reach, *ibid.*, **4**. See also Zunz, Annal. de la soc. roy. d. scienc. méd. et natur. de Bruxelles, **12** and **13**; Tobler, Zeitschr. f. physiol. Chem., **45**; London and Sulima, *ibid.*, **46**.

and that the protein foods are only prepared in the stomach for the real digestive processes in the intestine. That the stomach, at least the fundus part, serves in the first place as a storeroom follows from its shape, and this function is of special value in certain new-born animals, for instance in dogs and cats. In these animals the secretion of the stomach contains only hydrochloric acid but no pepsin, and the casein of the milk is converted by the acid alone into solid lumps or a solid coagulum which fills the stomach. Small portions of this coagulum pass into the intestine only little by little, and an overburdening of the intestine is thus prevented. In other animals, such as the snake and certain fishes which swallow their food entire, it is certain that the major part of the process of digestion takes place in the stomach. The importance of the stomach in digestion cannot at once be decided. It varies for different animals, and it may vary in the same animal, depending upon the division of the food, the rapidity with which the peptonization takes place, the more or less rapid increase in the amount of hydrochloric acid, and so on.

It is a well-known fact that the contents of the stomach may be kept without decomposing for some time by means of hydrochloric acid, while, on the contrary, when the acid is neutralized a fermentation commences by which lactic acid and other organic acids are formed. According to COHN an amount of hydrochloric acid more than 0.7 p. m. completely arrests lactic-acid fermentation, even under otherwise favorable circumstances, and according to STRAUSS and BIALOCOUR the limit of lactic-acid fermentation lies at 1.2 p. m. hydrochloric acid united to organic bodies. The hydrochloric acid of the gastric juice has unquestionably an antifermentative action, and also, like all dilute mineral acids, an antiseptic action. This action is of importance, as many pathogenic micro-organisms may be destroyed by the gastric juice. The common bacillus of cholera, certain streptococci, etc., are killed by the gastric juice, while others, especially as spores, are unacted upon. The fact that gastric juice can diminish or retard the action of certain toxalbumins, such as tetanotoxine and diphtheria toxine, is also of great interest (NENCKI, SIEBER, and SCHOUMOWA[1]).

Because of this antifermentative and antitoxic action of gastric juice it is considered that the chief importance of the gastric juice lies in its antiseptic action. The fact that intestinal putrefaction is not increased on the extirpation of the stomach, as derived from experiments made on man and animals,[2] does not uphold this view.

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[1] Cohn, Zeitschr. f. physiol. Chem., **14**; Strauss and Bialocour, Zeitschr. f. klin. Med., **28**. See also Kühne, Lehrb., **57**; Bunge, Lehrb. d. Physiol., 4. Aufl., 148 and 159; Hirschfeld, Pflüger's Arch., **47**; Nencki, Sieber, and Schoumowa, Centralbl. f. Bacteriol., etc., **23**. In regard to the action of gastric juice upon pathogenic microbes we must refer the reader to handbooks of bacteriology.

[2] See Carvallo and Pachon, l. c., and Schlatter in Wróblewski, l. c.

Since the hydrochloric acid of the gastric juice prevents the contents of the stomach from fermenting with the generation of gas, those *gases* which occur in the stomach probably depend, at least in great measure, upon the swallowed air and saliva, and upon those gases generated in the intestine and returned through the pyloric valve. PLANER found in the stomach-gases of a dog 66–68 per cent N, 23–33 per cent CO_2, and only a small quantity, 0.8–6.1 per cent, of oxygen. SCHIERBECK[1] has shown that a part of the carbon dioxide is formed by the mucous membrane of the stomach. The tension of the carbon dioxide in the stomach corresponds, according to him, to 30–40 mm. Hg in the fasting condition. It increases after partaking food, independently of the kind of food, and may rise to 130–140 mm. Hg during digestion. The curve of the carbon-dioxide tension in the stomach is the same as the curve of acidity in the different phases of digestion, and SCHIERBECK has also found that the carbon-dioxide tension is considerably increased by pilocarpine, but diminished by nicotine. According to him, the carbon dioxide of the stomach is a product of the activity of the secretory cells.

After death, if the stomach still contains food, autodigestion goes on not only in the stomach, but also in the neighboring organs, during the slow cooling of the body. This leads to the question, Why does the stomach not digest itself during life? Ever since PAVY has shown that after tying the smaller blood-vessels of the stomach of dogs the corresponding part of the mucous membrane was digested, efforts have been made to find the cause in the neutralization of the acid of the gastric juice by the alkali of the blood. That the reason for the non-digestion during life is to be sought for in the normal circulation of the blood cannot be contradicted; but the reason is not to be found in the neutralization of the acid. The investigations of FERMI and OTTE[2] show that the blood circulation acts in an indirect manner by the normal nourishment of the cell protoplasm, and this is the reason why the digestive fluids, the gastric juice as well as the pancreatic juice, act differently upon the living protoplasm as compared with the dead. We know nothing about this resistance of the living protoplasm. Some claim that it is connected closely with the secretion of the antipepsin discovered by DANILEWSKY, HÄNSEL, and WEINLAND, but this is hard to understand. Undoubtedly bodies occur in the gastric mucosa which can inhibit the action of pepsin, but whether these bodies are of an enzymotic nature or not is undecided. WEINLAND's antipepsin is related to the enzymes because it is thermolabile, while the antipepsin of DANILEWSKY,

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[1] Planer, Wien. Sitzungsber., **42**; Schierbeck, Skand. Arch. f. Physiol., **3** and **5**.

[2] Pavy, Phil. Transactions, **153**, part I, and Guy's Hospital Reports, **13**; Otte, Travaux du laboratoire de l'Institut de Physiol. de Liège, 5, 1896, which also contains the literature.

HÄNSEL, and O. SCHWARZ[1] is resistant towards heat and can hardly be considered as an enzymotic body. This is true for at least the thermostabile antipepsin of SCHWARZ, which does not give the biuret reaction. Without mentioning the still unknown nature of these bodies, the natural gastric juice, as well as an acid infusion of the mucosa, has such a strong digestive action that the retarding action of the antipepsin can only be shown under special conditions, and it is therefore difficult to conceive how the antipepsin could have a protective action in life.

Under pathological conditions, irregularities in the secretion as well as in the absorption and in the mechanical work of the stomach may occur. Pepsin is almost always present, although the amount may vary considerably, but the absence of the rennin, as above stated, may occur in many cases. In regard to the acid, we must remark that the secretion is sometimes increased so that an abnormally acid gastric juice is secreted and at other times it may be diminished so that little if any hydrochloric acid is formed. A hypersecretion of acid gastric juice sometimes occurs. In the secretion of too little hydrochloric acid the same conditions appear as after the neutralization of the acid contents of the stomach outside of the organism. Fermentation processes now appear in which, besides lactic acid, there occur also volatile fatty acids, such as butyric and acetic acids, etc., and gases like hydrogen. These fermentation products are therefore often found in the stomach in cases of chronic catarrh of the stomach, which may give rise to belching, pyrosis, and other symptoms.

Among the foreign substances found in the contents of the stomach we have UREA, or ammonium carbonate derived therefrom in uræmia; BLOOD, which generally forms a dark-brown mass through the presence of hæmatin, due to the action of the gastric juice; BILE, which, especially during vomiting, easily finds its way through the pylorus into the stomach, but whose presence seems to be without importance.

If it is desired to test the gastric juice or the contents of the stomach for *pepsin*, fibrin may be employed. If this is thoroughly washed immediately after beating the blood, well pressed, and placed in glycerine, it may be kept in serviceable condition for an indefinitely long time. The gastric juice or the contents of the stomach—the latter, if necessary, having been previously diluted with 1 p. m. hydrochloric acid—is filtered and tested with fibrin at ordinary temperature. (It must not be forgotten that a control test must be made with acid alone and another portion of the same fibrin.) If the fibrin is not noticeably digested within one or two hours, no pepsin is present, or at most there are only slight traces.

In testing for *rennin* the liquid must be first carefully neutralized. To 10 c.c. of unboiled, amphoteric (not acid) cow's milk add 1–2 c.c. of the filtered neutralized liquid. In the presence of rennin the milk should coagulate to a solid mass at the temperature of the body in the course of 10–12 minutes without changing its reaction. If the milk is diluted too much by the addition of the liquid of the stomach, only coarse flakes are obtained and no solid coagulum. Addition of lime-salts is to be avoided, as in great excess they may produce a partial coagulation even in the absence of typical rennin.

In many cases it is especially important to determine the *degree of*

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[1] See Hänsel, Biochem. Centralbl., **1**, p. 404, and **2**, p. 326; Weinland, Zeitschr. f. Biologie, **44**; Schwarz, Hofmeister's Beiträge, 6.

acidity of the gastric juice. This may be done by the ordinary titration methods. Phenolphthalein must not be used as an indicator, as too high results are produced in the presence of large quantities of proteins. Good results may be obtained, on the contrary, by using very delicate litmus paper. Although the acid reaction of the contents of the stomach may be caused simultaneously by several acids, still the degree of acidity is here, as in other cases, expressed in only one acid, e.g., HCl. Generally the acidity is designated by the number of cubic centimetres of N/10 caustic soda which is required to neutralize the several acids in 100 c.c. of the liquid of the stomach. An acidity of 43 per cent means that 100 c.c. of the liquid of the stomach required 43 c.c. of N/10 caustic soda to neutralize it.

It is also important to be able to ascertain the nature of the acid or acids occurring in the contents of the stomach. For this purpose, and especially for the *detection of free hydrochloric acid*, a great number of color reactions have been proposed which are all based upon the fact that the coloring substance gives a characteristic color with very small quantities of hydrochloric acid, while lactic acid and the other organic acids do not give these colorations, or only in a certain concentration, which can hardly exist in the contents of the stomach. These reagents are a mixture of FERRIC-ACETATE and POTASSIUM-SULPHOCYANIDE solution (MOHR's reagent has been modified by several investigators), METHYLANILINE-VIOLET, TROPÆOLIN 00, CONGO RED, MALACHITE-GREEN, PHLOROGLUCIN-VANILLIN, DIMETHYLAMINOAZOBENZENE, and others. As reagents for *free lactic acid* UFFELMANN suggests a strongly diluted, amethyst-blue solution of FERRIC CHLORIDE and CARBOLIC ACID or a strongly diluted, nearly colorless solution of FERRIC CHLORIDE. These give a yellow color with lactic acid, but not with hydrochloric acid or with volatile fatty acids.

The value of these reagents in testing for free hydrochloric acid or lactic acid is still disputed. Among the reagents for free hydrochloric acid GÜNZBURG's test with phloroglucin-vanillin, and the test with tropæolin 00, performed at a moderate temperature as suggested by BOAS, and the test with dimethylaminoazobenzene, which is the most delicate, seem to be the most valuable. If these tests give positive results, then the presence of hydrochloric acid may be considered as proved. A negative result does not eliminate the presence of hydrochloric acid, as the delicacy of these reactions has a limit, and also the simultaneous presence of protein, peptones, and other bodies influences the reactions more or less. The reactions for lactic acid may also give negative results in the presence of comparatively large quantities of hydrochloric acid in the liquid to be tested. Sugar, sulphocyanides, and other bodies may act with these reagents similarly to lactic acid.

In testing for lactic acid it is safest to shake the material with ether and test the residue after the evaporation of the solvent. On the evaporation of the ether the residue may be tested in several ways. BOAS[1] utilizes the property possessed by lactic acid of being oxidized into aldehyde and formic acid on careful oxidation with sulphuric acid and manganese dioxide. The aldehyde is detected by its forming iodoform with an alkaline iodine solution or by its forming aldehyde mercury with NESSLER's reagent.

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[1] **Deutsch.** med. Wochenschr., 1893, and Münchener med. Wochenschr, **1893.**

The quantitative estimation consists in the formation of iodoform with N/10 iodine solution and caustic potash, adding an excess of hydrochloric acid and titrating with a N/10 sodium-arsenite solution, and retitrating with iodine solution, after the addition of starch-paste, until a blue coloration is obtained. This method presupposes the use of ether entirely free from alcohol.

In testing for lactic acid CRONER and CRONHEIM[1] recommend a solution of iodine in potassium iodide containing aniline. Lactic acid is converted into iodoform, which with the aniline develops the nauseating odor of isonitrile. The shaking out of the lactic acid with ether is unnecessary, but naturally alcohol or acetone must not be added.

In order to be able to judge correctly of the value of the different reagents for free hydrochloric acid, it is naturally of greatest importance to be clear in regard to what we mean by free hydrochloric acid. It is a well-known fact that hydrochloric acid combines with proteins. and a considerable part of the hydrochloric acid may therefore exist in the contents of the stomach, after a meal rich in proteins, in combination with them. This hydrochloric acid combined with proteins cannot be considered as free, and it is for this reason that certain investigators consider such methods as that of SJÖQVIST, which will be described below. as of little value. However, it must be remarked that, according to the unanimous experience of many investigators, the hydrochloric acid combined with proteins is physiologically active. Those reactions (color reactions) which respond only to actually free hydrochloric acid do not show the physiologically active hydrochloric acid. The suggestion of determining the "physiologically active" hydrochloric acid instead of the "free" seems to be correct in principle; and as the conceptions of free and of physiologically active hydrochloric acid are not the same, it must always be well defined whether one wishes to determine the actually free or the physiologically active hydrochloric acid before any conclusions are drawn as to the value of a certain reaction.

The acid reaction may be partly due to free acid, partly to acid salts (monophosphates), and partly to both. According to LEO[2] one can test for acid phosphates by calcium carbonate, which is not neutralized therewith, while the free acids are. If the gastric content has a neutral reaction after shaking with calcium carbonate and the carbon dioxide is driven out by a current of air, then it contains only free acid; if it has an acid reaction, then acid phosphates are present, and if it is less acid than before, it contains both free acid and acid phosphate. It must not be forgotten that a faint acid reaction may, after treatment with calcium carbonate, also be due to the protein. This method can likewise be applied in the estimation of free acid.

Various titration methods have been suggested for the estimation of the free hydrochloric acid, but these cannot yield conclusive results for the reasons given in a previous chapter (see estimation of the alkalinity of the blood-serum, page 190). For this determination physico-chemical methods are necessary, but they have not been used to any great extent for clinical purposes on account of the difficulty in their manipulation.

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[1] Berlin. klin. Wochenschr., 1905, p. 1080.

[2] Centralbl. f. d. med. Wissensch., 1889, p. 481; Pflüger's Arch., 48, and Berlin. klin. Wochenschr., 1905, p. 1491.

As it is not within the scope of this book to give the various methods for the quantitative estimation of hydrochloric acid for clinical purposes we must refer to the various handbooks for clinical methods, such as those of v. JAKSCH, EULENBURG, KOLLE, and WEINTRAUD, and the work of O. REISSNER,[1] for details as to the qualitative and quantitative tests for hydrochloric acid and lactic acid.

The methods suggested by LEO, HAYEM and WINTER, MARTIUS and LÜTTKE, and by REISSNER, as well as the following method of MÖRNER and SJÖQVIST,[2] are used for the quantitative estimation of the total hydrochloric acid.

The method of K. MÖRNER and SJÖQVIST depends upon the following principle: When the gastric juice is evaporated to dryness with barium carbonate and then calcined, the organic acids burn up and give insoluble barium carbonate, while the hydrochloric acid forms soluble barium chloride. From the quantity of this the original amount of hydrochloric acid can be calculated. 10 c.c. of the filtered contents of the stomach are mixed in a small platinum or silver dish with a knife-point of barium carbonate free from chlorides and evaporated to dryness. The residue is burned and allowed to glow for a few minutes. The cooled carbon is gently rubbed with water and completely extracted with boiling water and the filtrate (about 50 c.c.) precipitated by ammonium chromate after the addition of ammonium acetate and acetic acid and boiling. The carefully collected precipitate is washed and dissolved in water by the aid of a little HCl, treated with KI, and hydrochloric acid and titrated with hyposulphite solution. The reactions take place as follows· $4HCl + 2BaCO_3 = 2BaCl_2 + {}_2H_2O + 2CO_2$; $2BaCl_2 + 2(NH_4)_2CrO_4 = 2BaCrO_4 + 4NH_4Cl$; $2BaCrO_4 + 16HCl + 6KI = 2BaCl_2 + Cr_2Cl_6 + 8H_2O + 6KCl + 3I_2$; and $3I_2 + 6Na_2S_2O_3 = 6NaI + 3Na_2S_4O_6$. Each cubic centimetre of the hyposulphite corresponds to 3 mgm. HCl. Complete directions for the necessary solutions and for the performance of the method may be found in SJÖQVIST, Zeitschr. f. klin. Med., 32.

In testing for *volatile fatty acids* the contents of the stomach should not be directly distilled, as volatile fatty acids may be formed by the decomposition of other bodies, such as protein and hæmoglobin. The neutralized contents of the stomach are therefore precipitated with alcohol at ordinary temperature, filtered quickly, pressed, and repeatedly extracted with alcohol. The alcoholic extracts are made faintly alkaline by soda and the alcohol distilled. The residue is now acidified by sulphuric or phosphoric acid and distilled. The distillate is neutralized by soda and evaporated to dryness on the water-bath. The residue is extracted with absolute alcohol, filtered, the alcohol distilled off, and this residue dissolved in a little water. This solution may be directly tested for acetic acid with sulphuric acid and alcohol or with ferric chloride. Formic acid may be tested for by silver nitrate, which quickly gives a black precipitate; and butyric acid is detected by the odor after the addition of an acid. In regard to the methods for more fully investigating the different volatile fatty acids, the reader is referred to other text-books.

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[1] Zeitschr. f. klin. Med., 48.

[2] In regard to the methods here mentioned see Reissner, l. c.

III. The Glands of the Mucous Membrane of the Intestine and their Secretions.

The Secretion of Brunner's Glands. These glands are partly considered as small pancreatic glands and partly as mucous or salivary glands. Their importance in various animals is different. According to GRÜTZNER they are closely related in dogs to the pyloric glands and contain pepsin. This also coincides with the observations of GLAESSNER and of PONOMAREW, which differ from each other only in that PONOMAREW finds that the secretion is inactive in alkaline reaction and contains only pepsin, while GLAESSNER claims it is active in both acid and alkaline reaction and that it contains propepsin. According to ABDERHALDEN and RONA[1] the pure duodenal secretion of the dog contains a proteolytic enzyme which does not belong to the trypsin type but rather to the pepsin variety. The statements as to the occurrence of a diastatic enzyme in BRUNNER's glands are disputed.

The Secretion of Lieberkuhn's Glands. The secretion of these glands has been studied by the aid of a fistula in the intestine according to the method of THIRY and VELLA. Very little if any secretion takes place in fasting animals (dogs) when the mucous membrane is not irritated. In lambs PREGL found the secretion continuous. The ingestion of food causes a secretion, and in lambs increases the secretion already taking place. Mechanical, chemical, and electrical stimulants act in the same manner in dogs (THIRY). The secretion is also markedly increased in man by the local irritation of the mucous membrane (HAMBURGER and HEKMA[2]). In the cases observed by these experimenters the flow of fluid was greatest at night as well as between five and eight o'clock in the afternoon, and was lowest between two and five o'clock in the afternoon. Pilocarpine does not increase the secretion in lambs, and in dogs it does not seem to be always active (GAMGEE[3]). Among the chemical excitants we must specially mention acids and gastric juice, which latter acts by its acidity. The action of acids seems to be indirect, by means of the secretin which will be mentioned below. Several salts, NaCl, Na_2SO_4, and others, may cause an abundant secretion of fluid into the intestine when injected intravenously or subcutaneously, as well as after direct application to the peritoneal surface of the intestine. This action can be arrested by the antagonistic, inhibiting action of a lime salt (MacCallum[1]). The quantity of this secretion in the course of twenty-four hours has not been exactly determined.

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[1] Grützner, Pflüger's Arch., 12; Glaessner, Hofmeister's Beiträge, 1; Ponomarew, Biochem. Centralbl., 1, 351; Abderhalden and Rona, Zeitschr. f. physiol. Chem., 47.

[2] Thiry, Wien. Sitzungsber., 50; Vella, Moleschott's Untersuch., 13; Pregl, Pflüger's Arch., 61; Gamgee, Physiol. Chem., 2, 410, where Vella and Masloff are quoted; Krüger, Zeitschr. f. Biologie, 37; Hamburger and Hekma, Journ. de physiol. et de path. générale, 1902 and 1904.

[3] Gamgee, l. c.

According to Delezenne and Frouin, if any mechanical irritation is prevented, the fluid flowing spontaneously from a fistula in a dog is ten times more abundant in the duodenum than that in the middle or lower part of the jejunum. In the upper part of the small intestine of the dog, on the contrary, this secretion is scanty, slimy, and gelatinous; in the lower part it is more fluid, with gelatinous lumps or flakes (Röhmann). Intestinal juice has a strong alkaline reaction towards litmus, generates carbon dioxide on the addition of an acid, and contains (in dogs) nearly a constant quantity of NaCl and Na_2CO_3, 4.8–5 and 4–5 p. m. respectively (Gumilewski, Röhmann[2]). The intestinal juice of the lamb corresponded to an alkalinity of 4.54 p. m. Na_2CO_3. It contains protein (Thiry found 8.01 p. m.), the quantity decreasing with the duration of the elimination. The quantity of solids varies. In dogs the quantity of solids is 12.2–24.1 p. m. and in lambs 29.85 p. m. The specific gravity of the intestinal juice of the dog, according to the observations of Thiry, is 1.010–1.0107, and in lambs 1.01427 (Pregl). The intestinal juice from lambs contains 18.097 p. m. protein, 1.274 p. m. proteoses and mucin, 2.29 p. m. urea, and 3.13 p. m. remaining organic bodies.

We have the investigations of Demant, Turby and Manning, H. Hamburger and Hekma and Nagano[3] on the human intestinal juice. Human intestinal juice has a low specific gravity, nearly 1.007, about 10–14 p. m. solids, and is strongly alkaline towards litmus. The content of alkali calculated as sodium carbonate is 2.2 p. m., according to Nagano, Hamburger and Hekma, and 5.8–6.7 p. m. NaCl. The determination of the freezing-point was −0.62° (Hamburger and Hekma).

The intestinal juice of the dog contains, according to Boldireff,[4] a *lipase* which acts especially upon emulsified fat (milk) and is different from pancreas lipase. The intestinal juice of animals and man also contains an enzyme, *erepsin*, discovered by O. Cohnheim, which does not act ordinarily upon native proteins, but upon proteoses and peptones, and the juice also has a faint amylolytic action. The juice, and to a high degree the mucous coat, contains *invertase* and *maltase*, which fact has been recently substantiated by the observations of Paschutin, Brown and Heron, Bastianelli, and TEBB.[1] A lactose-inverting enzyme, a *lactase*, also occurs, as shown by RÖHMANN and LAPPE, PAUTZ and VOGEL, WEINLAND, and ORBAN,[2] in new-born infants and young, animals, and also in grown mammals which were fed upon a milk diet. The lactase is found to a greater extent in the mucosa than in the juice.

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[1] University of California Publications, **1**, 1904.

[2] Delzenne and Frouin, Compt. rend. soc. biolog., **56**; Gumilewski, Pflüger's Arch., **39**; Röhmann, *ibid.*, **41**.

[3] Demant, Virchow's Arch., **75**; Turby and Manning, Centralbl. f. d. med. Wissenschaft, 1892, 945; Hamburger and Hekma, l. c.; Nagano, Mitt. aus d. Grenzgeb. d. Med. u. Chir., **9**.

[4] Boldireff, Archiv d. sciences biolog. de St. Pétersbourg, **11**.

Besides erepsin and the other enzymes mentioned the intestinal mucosa also contains antienzymes, *antipepsin* and *antitrypsin* (DANILEWSKY and WEINLAND[3]), also *enterokinase* or a mother-substance of the same, and finally also the so-called *prosecretin*. These two last-mentioned bodies, which are closely connected with the secretion of pancreatic juice, will be discussed in connection with this digestive fluid.

The various enzymes are not formed in equal quantities in all parts of the intestine. Lipase, diastase, and invertase occur, according to BOLDIREFF, all through the intestine, while the kinase occurs only in the upper part of the intestine (BOLDIREFF, BAYLISS and STARLING, DELEZENNE). According to HEKMA the kinase occurs in all parts of the intestine, but most abundantly in the duodenum and the upper part of the jejunum. The enzymes, according to FALLOISE, generally occur in greatest abundance in the upper parts of the intestine; but the erepsin occurs to a greater extent in the jejunum than in the duodenum. According to the investigations of VERNON the behavior of erepsin in different animals is not the same. In cats and hedge-hogs the duodenum is richer in erepsin than the jejunum and ileum; in rabbits it is the reverse, namely, the ileum is much richer than the duodenum. The secretion, according to BAYLISS and STARLING, is formed entirely in the upper part of the intestine. The epithelium-cells of the glands or the mucous membrane are generally considered as the seat of formation of the enzymes, and the same is true also for the enterokinase, according to BAYLISS and STARLING, HEKMA, FALLOISE, and others, which, however, according to DELEZENNE,[4] is formed in the leucocytes and PEYER's glands.

BOTTAZZI[5] has obtained a very complex protein from the intestinal mucosa, which is readily soluble in water and alkali but is precipitated by acids. It coagulates at 55° to 56° and probably also contains carbohydrate and considerable iron. Intravenous injection of this protein brings about an abundant secretion of saliva, pancreatic juice, bile, and intestinal juice, and promotes the peristaltic movements of the intestine.

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[1] Paschutin, Centralbl. f. d. med. Wissensch., 1870, 561; Brown and Heron, Annal. d. Chem. u. Pharm., **204**; Bastianelli, Moleschott's Untersuch. zur Naturlehre, **14** (this contains all the older literature). See also Miura, Zeitschr. f. Biologie, **32**; Widdicombe, Journ. of Physiol., 28; Tebb, *ibid.*, **15**.

[2] Röhmann and Lappe, Ber. d. deutsch. chem. Gesellsch., 28; Pautz and Vogel Zeitschr. f. Biologie, 32; Weinland, *ibid.*, 38; Orban, Maly's Jahresber., **29**.

[3] See foot-note 1, p. 373.

[4] Boldireff, Arch. d. scienc. biolog. de St. Pétersbourg, **11**; Bayliss and Starling, Journ. of Physiol., **29**, **30**; Hekma, l. c.; Falloise, see Biochem. Centralbl., **4**, p. **153**; Vernon, Journ. of Physiol., **33**; Delezenne, Compt. rend. soc. biolog **54** and **56**.

[5] See Biochem. Centralbl., **3**, p. 65.

Erepsin. This enzyme, discovered by O. COHNHEIM, has no direct action upon native proteins with the exception of casein, but has the power of splitting proteoses and peptones. In this change mono- as well as di-amino-acids are produced. Erepsin occurs in the mucous membrane and in the intestinal juice of man as well as of dogs; the mucous membrane seems to be richer than the juice (SALASKIN, KUTSCHER and SEEMANN[1]). An enzyme like erepsin occurs also in the pancreas (BAYLISS and STARLING, VERNON), and this has the power of acting upon casein, but not, or only faintly, upon fresh fibrin. This erepsin is probably identical with the enzyme *nuclease*, discovered by F. SACHS in the pancreas, which acts upon nucleic acids, while NAKAYAMA claims that erepsin differs from trypsin by having a cleavage action upon nucleic acids. Erepsin shows a great similarity to the intracellular enzymes active in autolysis, and according to VERNON erepsins occur in the various tissues of invertebrates as well as vertebrates. These tissue erepsins, which are closely related to the autolytic enzymes, if they are not identical, behave somewhat differently from the intestinal erepsin and are not identical therewith. Enzymes having an action similar to erepsin occur, according to VINES,[2] in all plants so far investigated.

Erepsin becomes inactive on heating to 59°. It works best in alkaline solution, but has hardly any action in faint acid reaction. In this regard, as well as by the fact that only a little ammonia is split off by its action upon peptone substances, it differentiates itself from certain of the autolytic enzymes studied so far.

The secretion of the **glands in the large intestine** seems to consist chiefly of mucus. Fistulas have also been introduced into these parts of the intestine, which are chiefly, if not entirely, to be considered as absorption organs. The investigations on the action of this secretion on nutritive bodies have not as yet yielded any positive results.

IV. The Pancreas and Pancreatic Juice.

In invertebrates, which have no pepsin digestion and which also have no formation of bile, the pancreas, or at least an analogous organ, seems to be the essential digestive gland. On the contrary, an anatomically characteristic pancreas is absent in certain vertebrates and in certain fishes.

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[1] Cohnheim, Zeitschr. f. physiol. Chem., **33**, **35**, **36**, and **47**; Salaskin, *ibid.*, **35**; Kutscher and Seemann, *ibid.*, **35**.

[2] Bayliss and Starling, Journ. of Physiol., **30**; Vernon, *ibid.*, **30** and **33**; F. Sachs, Zeitschr. f. physiol. Chem., **46**; Nakayama, *ibid.*, **41**; Vines, Annals of Botany, **18** and **19**.

Those functions which should be regulated by this organ seem to be performed in these animals by the liver, which may be rightly called the HEPATOPANCREAS. In man and in most vertebrates the formation of bile and of certain secretions containing enzymes important for digestion is divided between the two organs, the liver and the pancreas.

The **pancreatic gland** is similar in certain respects to the parotid gland. The secreting elements of the former consist of nucleated cells whose basis forms a mass rich in proteins, which expands in water and in which two distinct zones exist. The outer zone is more homogeneous, the inner cloudy, due to a quantity of granules. The nucleus lies about midway between the two zones, but this position may change with the varying relative size of the two zones. According to HEIDENHAIN[1] the inner part of the cells diminishes in size during the first stages of digestion, in which the secretion is active, while at the same time the outer zone enlarges owing to the absorption of new material. In the later stage, when the secretion has decreased and the absorption of the nutritive bodies has taken place, the inner zone enlarges at the expense of the outer, the substance of the latter having been converted into that of the former. Under physiological conditions the glandular cells are undergoing a constant change, at one time consuming from the inner part and at another time growing from the outer part. The inner granular zone is converted into the secretion, and the outer, more homogeneous zone, which contains the repairing material, is then converted into the granular substance. The so-called islands of LANGERHANS are related to the internal secretion or contain a substance taking part in the transformation of the sugar of the animal body.[2]

The chief portion of protein substances contained in the gland consists, it seems, of *nucleoproteids*, while the *globulins* and *albumins* occur only to a slight extent as compared with the nucleoproteids. Among the compound proteids is the substance studied and isolated by UMBER but previously discovered by HAMMARSTEN[3] and called α-proteid. This nucleoproteid contains, as an average, 1.67 per cent P, 1.29 per cent S, 17.12 per cent N, and 0.13 per cent Fe. It yields on boiling β-proteid, so called by HAMMARSTEN, which is much richer in phosphorus than the nucleoproteid. The native proteid (α) is the mother-substance of guanylic acid; according to UMBER it dissolves by pepsin digestion without leaving any residue and yields on trypsin digestion guanylic acid on one side and proteoses and peptones on the other. It can be extracted from the gland by a physiological salt solution and is precipitated by acetic acid. Besides this compound proteid the pancreas must contain at least one other proteid, which is the mother-substance of the thymonucleic acid obtainable from the pancreas.

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[1] Pflüger's Arch., 10.

[2] See Diamare and Kuliabko, Centralbl. f. Physiol., 18 and 19; Rennie, *ibid.*, 18; Sauerbeck, Virchow's Arch.. 177 Suppl.

[3] Umber, Zeitschr. f. klin. Med., 40 and 43; Hammarsten, Zeitschr. f. physiol. Chem., 19.

Besides these protein substances the gland contains also several enzymes, or more correctly *zymogens,* which will be discussed later. Among the extractive bodies, which are probably in part formed by post-mortem changes and chemical action, we must mention *leucine* (butalanine), *tyrosine, purine bases* in variable quantities,[1] *inosite, lactic acid, volatile fatty acids,* and *fats.* The mineral bodies vary considerably in quantity not only in animals and man but also in men and women (GOSSMANN). The calcium seems, according to GOSSMANN, to exist in much greater amount than the magnesium. According to the investigations of OIDTMANN the pancreas of an old woman contains 745.3 p. m. water, 245.7 p. m. organic and 9.5 p. m. inorganic substances. GOSSMANN[2] found in a man 17.92 p. m. ash and 13.05 p. m. in a woman.

Besides the already-mentioned (Chapter VIII) relationship to the transformation of sugar in the animal body, the pancreas has the property of secreting a juice especially important in digestion.

Pancreatic Juice. This secretion may be obtained by adjusting a fistula in the excretory duct, according to the methods suggested by BERNARD, LUDWIG, and HEIDENHAIN, and perfected by PAWLOW.[3] If the operation is performed with sufficient rapidity and under favorable conditions a powerfully active secretion may be obtained either immediately after the operation (*temporary fistula*) or after some time (*permanent fistula*).

In herbivora, such as rabbits, whose digestion is uninterrupted, the secretion of the pancreatic juice is continuous. In carnivora it seems, on the contrary, to be intermittent and dependent on the digestion. During starvation the secretion almost stops, but commences again after partaking of food and reaches its maximum, according to BERNSTEIN, HEIDENHAIN, and others, within the first three hours. According to PAWLOW and his school (WALTHER[4]) this maximum is dependent upon the character of the food. With milk diet it appears within three to four hours, after bread diet at the end of the second hour, and with a meat diet it arrives still sooner. The quality of the juice is also, according to PAWLOW's school, dependent upon the food, and the amount of the three enzymes, diastase, trypsin, and steapsin, changes with the variety of food. The observations which form the basis of this view have been somewhat differently explained in the light of recent investigations on the conditions necessary for the conversion of trypsinogen into trypsin.

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[1] See Kossel, Zeitschr. f. physiol. Chem., 8.

[2] Gossmann, Maly's Jahresber., **30**; Oidtmann, cited from Gorup-Besanez, Lehrbuch, 4th Ed., 732.

[3] Bernard, Leçons de Physiol., **2**, 190; Ludwig, see Bernstein, Arbeiten a. d. physiol. Anstalt zu Leipzig, 1869; Heidenhain, Pflüger's Arch., **10**, 604; Pawlow, Die Arbeit der Verdauungsdrsüen, Wiesbaden, 1898, and Ergebnisse der Physiologie, **1**, Abt. 1.

[4] Bernstein, l. c., foot-note 3, Walther, Arch. des sciences biol. de St. Pétersbourg, **7**.

Pawlow and his pupils, especially Schepowalnikoff, have shown that the above-mentioned (page 379) enterokinase activates the trypsinogen into trypsin. These observations were later confirmed by others, especially by Delezenne and Frouin, Popielski, Camus and Gley, Bayliss and Starling, and further studied. The pure juice contains only trypsinogen and no trypsin. By mixing with the intestinal juice, or by contact with the intestinal mucosa, the trypsinogen is converted into trypsin by the kinase. Enterokinase, which itself has no action upon proteins, has been found in all higher animals examined. A kinase with a similar action has also been detected by Delezenne in the lymph-glands and in impure fibrin, a statement which is contradicted by Bayliss and Starling and Hekma. The enterokinase is made inactive by heat and is therefore considered as an enzyme. Hamburger and Hekma, who detected enterokinase in human intestinal juice, do not consider it an enzyme, because a certain quantity of intestinal juice will activate only a certain quantity of trypsin (see below).

The above statements concerning the action of a varying diet upon the enzyme content of the juice have been somewhat changed by the investigations of Pawlow's school (Lintwarew and others). For instance, a diet of bread and milk causes the secretion of a large quantity of juice which is rich in trypsinogen but contains almost no trypsin. On giving meat after this the juice also contains trypsin; after a rich meat diet the secretion becomes scant and the juice contains only trypsin but no trypsinogen. There is here one difference between Pawlow's school and certain other investigators. According to Delezenne and Frouin, Popielski, Bayliss and Starling, Prym, and others,[1] the juice never contains trypsin but always only trypsinogen, if it is collected through a canula in Wirsung's duct, so that contact with the intestinal mucosa is prevented. Popielski explains the observations of Pawlow's school by the fact that a contact of the juice with the intestinal secretions was not perfectly prevented, and that with one kind of diet a rapid flow of juice took place and with another a slower flow.

It is not clear whether there are also kinases for the other two enzymes. Pawlow's pupils claim that the diastase is always eliminated as enzyme, while according to Pozerski a kinase also exists for this zymogen. In regard to steapsin the statements are somewhat contradictory. According to Lintwarew there is secreted with food rich in carbohydrates and fats a zymogen which is quickly changed into the enzyme by bile or intestinal juice. With a meat diet the steapsin is secreted already formed.

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[1] In regard to the literature on enterokinase, secretin, and secretion of pancreatic juice, see O. Cohnheim, Biochem. Centralbl., 1, 169, and S. Rosenberg, *ibid.*, 2, 708; Prym, Pflüger's Arch., 104 and 107.

The specific irritants for the secretion of pancreatic juice are, according to Pawlow and his collaborators, acids of various kinds—hydrochloric acid as well as lactic acid—and fats, the latter acting probably by virtue of the soaps produced therefrom. Alkalies and alkali carbonates have, on the contrary, a retarding action. It appears that the acids act by irritating the mucosa of the duodenum. Water, which causes a secretion of acid gastric juice, likewise becomes indirectly a stimulant for the pancreatic secretion, but may also be an independent exciter. The psychical moment may, at least in the first place, have an indirect action (secretion of acid gastric juice), and the food seems otherwise to have an action on pancreatic secretion by its action on the secretion of gastric juice.

The most important excitant for the secretion of juice is hydrochloric acid, but the views are not united as to the manner in which the acid acts According to Pawlow's school, the acid acts reflexly upon the intestine, causing a secretion of a juice containing only trypsinogen. That a reflex action is here produced is not contradicted by the investigations of Popielski, Wertheimer and Lepage, Fleig,[1] and others. According to the researches of Bayliss and Starling, which have been confirmed by Camus, Gley, Fleig, Herzen, Delezenne, and others, a second factor must also be active here. Bayliss and Starling have shown that a body which they have called *secretin* can be extracted from the intestinal mucosa by a hydrochloric-acid solution of 4 p. m., and this when introduced into the blood produces a secretion of pancreatic juice. The secretin, which according to Bayliss and Starling [2] is the same in all vertebrates examined, is not destroyed by heat; it is therefore not identical with enterokinase, and is not considered as an enzyme. It is formed from another substance, *prosecretin*, by the action of acids. According to Delezenne and Pozerski[3] secretin occurs as such in the intestinal mucosa, and the acid acts only by the destruction of certain bodies having a retarding action. According to Popielski secretin action is different from acid action; the secretin according to him is a peptone, and the secretin action can also be obtained by Witte's peptone. The statements about secretin and its action are very divergent. It is difficult to obtain a clear conception of the amount of zymogens or enzymes secreted by the juice under the influence of the secretin. It seems to be clear that this juice, at least in many cases, contains only trypsinogen and no trypsin.

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[1] Centralbl. f. Physiol., **16**, 681, and Compt. rend. soc. biol., **55**. See also foot- 1, p. 383.

[2] Journ. of Physiol., **29**.

Delezenne and Pozerski, Compt. rend. soc. biol., **56**; Popielski, Centralbl. f. L, **19**.

A second means of causing secretion is the fat, which probably only acts after it has been saponified. Oil-soap introduced directly into the duodenum brings about a strong secretion of pancreatic juice (Sawitsch, Babkine 1)), and at the same time a flow of bile, gastric juice, and the secretion of Brunner's glands occurs. The pancreatic juice secreted under these circumstances has about the same amount of enzymes as the juice secreted after partaking of food. We know very little as to how the soaps act. Fleig[2] has found that by maceration of the mucosa of the upper part of the duodenum with soap solution a substance goes into solution, which he calls *sapokrinin* and which when introduced into the blood brings about a strong secretion of pancreatic juice. This sapokrinin, which is derived from a prosapokrinin, is not an enzyme and is not identical with secretin. It dissolves in 60 per cent alcohol and is not destroyed by boiling. Sapokrinin affects the secretion of pancreatic juice alone, while the soaps also excite the secretion of bile and gastric juice. The secretion of pancreatic juice may also be increased by alcohol (Fleig, Gizelt 3)).

The activation of the trypsinogen into trypsin may in life be brought about—as the researches of Herzen, which have been substantiated by Gachet and Pachon, Bellamy, Mendel and Rettger, have shown—not only in the intestine, but also in the gland itself. This activation of the trypsinogen in the gland itself is caused in a manner still unknown by a body of unknown nature formed in the spleen, which is congested during digestion. Such a "charging" of the pancreas by the spleen has been repeatedly suggested by Schiff,[4] but this has recently been denied by Prym. According to this experimenter the extirpation of the spleen causes no change in the properties of the pancreatic juice, and the intravenous injection of spleen infusion is also without action on a splenectomized dog with permanent pancreatic fistula. The observations of Herzen that a spleen infusion has a strong activating action upon a weak pancreas infusion were substantiated by Prym,[5] but he claims that this is due essentially to micro-organisms.

The conversion of the trypsinogen into trypsin in the removed gland or

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[1] Arch. des scienc. biolog. de St. Pétersbourg, 11.

Compt. rend. soc. biolog., 55, and Journ. de physiol. et de pathol. gén., 1904.

[3] Centralbl. f. Physiol., 19.

[4] Bellamy, Journ. of Physiol., 27; Mendel and Rettger, Amer. Journ. of Physiol., 7. A very complete reference to the literature may be found in Menia Besbokaia Du rapport fonctionell entre le pankréas et la rate, Lausanne, 1901.

[5] Pflüger's Arch., 104 and 10..

in an infusion under the influence of air and water and also by other bodies has been known for a long time. According to VERNON the trypsin itself has a strong activating action upon trypsinogen, and in this regard it is more active than enterokinase. The correctness of this statement is still denied by BAYLISS and STARLING and by HEKMA. The ordinary view of HEIDENHAIN, that the transformation of trypsinogen into trypsin is also brought about by acids, has been found to be incorrect by HEKMA.[1] Besides the enterokinase and the micro-organisms we know for the present of no agent of organic origin which has the power of activating trypsinogen with positiveness. According to DELEZENNE, on the contrary, the pancreatic juice can be activated by calcium salts, and according to E. ZUNZ[2] also by magnesium and in certain cases by barium, lithium, and strontium salts.

The way in which the trypsinogen is converted into trypsin is still unknown and is the subject of disputed views. According to one view, proposed by PAWLOW and defended by BAYLISS and STARLING, the trypsinogen is transformed into trypsin by the action of the kinase. According to the views of DELEZENNE, DASTRE and STASSANO, and others,[3] the trypsin, on the contrary, is a combination between the kinase and trypsinogen, analogous to the hæmolysins, which according to EHRLICH's side-chain theory are combinations between a complement and an amboceptor.

The reflex formation of lactase after the introduction of milk-sugar into the intestine, as observed by WEINLAND, is to be considered as an intraglandular enzyme formation. This is a special example of the general rule based upon BROCARD's researches, that the kind of food has a marked influence upon the formation of hydrolytic ferments in the body; "c'est l'aliment qui fait le ferment." It has not been determined in what way the milk-sugar produces this adaptation of the gland. The investigations of BAINBRIDGE[4] seem to show that the milk-sugar causes the production of a body in the intestinal mucosa, which is brought to the pancreas by the blood and there makes the formation of lactase possible. This special property of the pancreas is denied by PLIMMER.[5]

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[1] Vernon, Journ. of Physiol., **28**; Hekma, Kon. Akad. v. Wetenschappen te Amsterdam, 1903, and Arch. f. (Anat. u.) Physiol., 1904; Bayliss and Starling, Journ. of Physiol., **30**.

[2] Delezenne, Compt. rend. soc. biolog., **59**, and Compt. rend., **141**; Zunz, see Biochem. Centralbl., **5**, 69.

[3] Bayliss and Starling, Journ. of Physiol., **30** and **32**, which also cites the other investigators. See also foot-note 1, p. 383.

[4] Weinland, Zeitschr. f. Biologie, **38** and **40**; Brocard, Journ. de physiol. et de path. gén., **4**; Bainbridge, Journ. of Physiol., **31**. Contradictory views are given by Bierry, Compt. rend., **140**, and Compt. rend. soc. biolog., **58**, and Plimmer, Journ. of Physiol., **34**.

[5] Journ. of Physiol., **34**.

The statements as to the quantity of pancreatic juice secreted in the twenty-four hours differ very much. According to the determinations of PAWLOW and his collaborators, KUWSCHINSKI, WASSILIEW, and JABLONSKY,[1] the average quantity (with normally acting juice) from a permanent fistula in dogs is 21.8 c.c. per kilo in the twenty-four hours.

The pancreatic juice of the dog is a clear, colorless, and odorless alkaline fluid which when obtained from a temporary fistula is very rich in proteins, sometimes so rich that it coagulates like the white of the egg on heating. Besides *proteins* the juice contains also the three above-mentioned enzymes (or their zymogens), *amylopsin, trypsin, steapsin,* also an enzyme similar to erepsin, and besides these a *rennin,* which was first observed by KÜHNE. Besides the above-mentioned bodies the pancreatic juice habitually contains small quantities of *leucine, fat,* and *soaps.* As mineral constituents it contains chiefly alkali chlorides and considerable alkali carbonate, some phosphoric acid, lime, magnesia, and iron.

The older analyses of the juice from a permanent fistula by C. SCHMIDT are the results of a more or less abnormal secretion, hence we shall give only the analyses of juices from temporary fistulas on dogs.[2] The results are given in parts per 1000.

	a.	*b.*
Water	900.8	884.4
Solids	99.2	115.6
Organic substance	90.4	.....
Ash	8.8	.....

The mineral constituents consisted chiefly of NaCl, 7.4 p. m., which is remarkable because the juice contains such a large amount of alkali carbonate. In the juice examined by DE ZILWA[3] the quantity of alkali in the secretin juice was 5–7.9 p. m. and in the pilocarpin juice 2.9–5.3 p. m. Na_2CO_3.

In the pancreatic juice of rabbits 11–26 p. m. solids have been found, and in that from sheep 14.3–36.9 p. m. In the pancreatic juice of the horse 9–15.5 p. m. solids have been found; in that of the pigeon, 12-14 p. m.

The human physiological pancreatic secretion from a fistula has been investigated by GLAESSNER.[3] The secretion was clear, foamed readily, had a strong alkaline reaction even towards phenolphthalein, and contained globulin and albumin but no proteoses and peptones. The specific gravity was 1.0075 and the freezing-point depression was $\Delta = -0.46$–$0.49°$. The solids were 12.44–12.71 p. m., the total protein 1.28–1.74 p. m., and the mineral bodies 5.66–6.98 p. m. The secretion contained trypsinogen, which was activated by the intestinal juice. Diastase and lipase were present; inverting enzymes, on the contrary, were not. The daily quantity of juice was 500–800 c.c. The quantity of secretion, of ferments, and of alkalinity was lowest in starvation, but soon rose with the taking of food, and reached its maximum in about four hours.

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[1] Arch. des sciences de St. Pétersbourg, **2**, 391. The older statements of Keferstein and Hallwachs, Bidder and Schmidt, and others may be found in Kühne, Lehrbuch, 114.

[2] Cited from Maly in Hermann's Handbuch der Physiol., **5**, Theil II, 189.

[3] Journ. of Physiol., **31**.

[4] Zeitschr. f. physiol. Chem., **40**. See also Ellinger and Kohn, *ibid.*, **45**, and the investigations upon cystic fluids from the pancreas by Schumm, *ibid.*, **36**, and Murray and Gies, American Medicine, **4**, 1902.

Amylopsin or **pancreatic diastase,** which, according to Korowin and Zweifel, is not found in new-born infants and does not appear until more than one month after birth, seems, although not identical with ptyalin, to be nearly related to it. Amylopsin acts very energetically upon boiled starch, and according to Kühne also upon unboiled starch, especially at 37° to 40° C., and according to Vernon [1] best at 35° C. It forms, similar to the action of saliva, besides dextrin, chiefly isomaltose and maltose, with only very little dextrose (Musculus and v. Mering, Külz and Vogel [2]). The dextrose is probably formed by the action of the invertin existing in the gland and juice. The pancreatic juice of the dog contains in fact, according to Bierry and Terroine,[3] maltase, whose action becomes apparent only after very faint acidification of the juice. According to Rachford the action of the amylopsin is not reduced by very small quantities of hydrochloric acid, but is diminished by larger amounts. Vernon, Grützner, and Wachsmann [4] find that the action is indeed accelerated by very small quantities of hydrochloric acid, 0.045 p. m., while alkalies in very small amounts have a retarding action. This retarding action of alkalies and hydrochloric acid may be stopped by bile (Rachford.)

If the natural pancreatic juice is not to be obtained, then the gland may be treated with water or glycerine. This infusion or the glycerine extract diluted with water (when glycerine has been used which has no reducing action) may be tested directly with starch-paste. It is safer, however, to first precipitate the enzyme from the glycerine extract by alcohol, and wash with this liquid, dry the precipitate over sulphuric acid, and extract with water. The enzyme is dissolved by the water. The test for sugar may be performed in the same manner as in the saliva.

Steapsin or **Fat-splitting Enzyme.** The action of the pancreatic juice on fats is twofold. First, the neutral fats are split into fatty acids and glycerine, which is an enzymotic process; and secondly, it has also the property of emulsifying the fats.

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[1] Korowin, Maly's Jahresber., **3**; Zweifel, foot-note 1, p. 344; Kühne, Lehrbuch, 117; Vernon, Journ. of Physiol., **27**.

[2] See foot-note 4, p. 344.

[3] See Tebb, Journ. of Physiol., **15**; Bierry and Terroine, Compt. rend. soc. biolog. **58**.

[4] Rachford, Amer. Journ. of Physiol., **2**; Vernon, l. c.; Grützner, Pflüger's Arch., **91**.

The action of the pancreatic juice in splitting the fats may be shown in the following way: Shake olive-oil with caustic soda and ether, siphon off the ether and filter if necessary, then shake the ether repeatedly with water and evaporate at a gentle heat. In this way is obtained a residue of fat free from fatty acids, which is neutral and which dissolves in acid-free alcohol and is not colored red by alkanet tincture. If such fat is mixed with perfectly fresh alkaline pancreatic juice or with a freshly prepared infusion of the fresh gland and treated with a little alkali or with a faintly alkaline glycerine extract of the fresh gland (9 parts glycerine and 1 part 1 per cent soda solution for each gram of the gland), and some litmus tincture added and the mixture warmed to 37° C., the alkaline reaction will gradually disappear and an acid one take its place. This acid reaction depends upon the conversion of the neutral fats by the enzyme into glycerine and free fatty acids.

The splitting of the neutral fats may also be shown more exactly by the following method: The mixture of neutral fats (absolutely free from fatty acids) and pancreatic juice or pancreas infusion is digested at the temperature of the body and treated with some soda and repeatedly shaken with fresh quantities of ether until all the unsplit neutral fats are removed. Then it is made acid with sulphuric acid, and after the acid liquor has been shaken with ether, the ether is evaporated, and the residue tested for fatty acids.

Another simple process for the demonstration of the fat-splitting action of the pancreatic glands is the following (CL. BERNARD): A small portion of the perfectly fresh, finely divided gland substance is first soaked in alcohol (90 per cent). Then the alcohol is removed as far as possible by pressing between blotting-paper, after which the pieces of gland are covered with an ethereal solution of neutral butter-fat (which may be obtained by shaking milk with caustic soda and ether). After the evaporation of the ether the pieces of gland covered with butter-fat are pressed between two watch-glasses and then gently heated to 37° to 40° C. in this position. After some time a marked odor of butyric acid appears.

The action of the pancreatic juice in splitting fats is a process analogous to that of saponification, the neutral fats being decomposed, by the addition of the elements of water, into fatty acids and glycerine according to the following formula: $C_3H_5.O_3.R_3$ (neutral fat) $+3H_2O=C_3H_5.O_3.H_3$ (glycerine) $+3(H.O.R)$ (fatty acid). This depends upon a hydrolytic splitting, which was first positively proved by BERNARD and BERTHELOT. The pancreas enzyme also decomposes other esters, just as it does the neutral fats (NENCKI, BAAS). The fat-splitting enzyme of the pancreas is, according to PAWLOW and BRUNO, aided in its action by the bile, and according to ENGEL obeys SCHÜTZ-BORISSOW'S rule that the extent of cleavage during a given time is proportional to the square root of the quantity of ferment. The investigations of KANITZ[1] have led to the same results.

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[1] Bernard, Ann. de chim. et physique (3), **25**; Berthelot, Jahresber. d. Chem., 1855, 733; Nencki, Arch. f. exp. Path. u. Pharm., **20**; Baas, Zeitschr. f. physiol. Chem., **14**, 416; Bruno, Arch. des sciences biolog. de St. Pétersbourg, **7**; Engel, Hofmeister's Beiträge, **7**; Kanitz, Zeitschr. f. physiol. Chem., **46**.

Pottevin[1] found that the pancreas (free from water) could form olein from oleic acid and glycerine It is claimed that the gland can form other esters from oleic acid or stearic acid with other alcohols (amyl alcohol) if we operate only in the absence of water. In the presence of considerable water the pancreas has a reverse saponifying action.

The fatty acids which are split off by the action of the pancreatic juice combine in the intestine with the alkalies, forming soaps, which have a strong emulsifying action on the fats, and thus the pancreatic juice becomes of great importance in the emulsification and the absorption of the fats.

Trypsin. The action of the pancreatic juice in digesting proteins was first observed by Bernard, but first proved by Corvisart.[2] It depends upon a special enzyme called by Kühne trypsin. This enzyme, as previously explained, does not occur in the gland as such but as trypsinogen. According to Albertoni[3] this zymogen is found in the gland in the last third of the intra-uterine life. Enzymes more or less like trypsin occur in other organs and are very widely diffused in the vegetable kingdom,[4] in yeast and in higher plants, and are also formed by various bacteria.

As we know of so-called antienzymes for other enzymes, so we also have antitrypsins and not only in the intestinal canal but also in the blood-serum. The results as to the specificity of these antitrypsins in various animals, as well as the possibility of producing antitrypsins by immunization, are still disputed

Trypsin, like other enzymes, has not been prepared in a pure condition. Nothing is positively known in regard to its nature, but as obtained thus far it shows a variable behavior (Kühne, Klug, Levene, Mays, and others). At least it does not seem to be a nucleoproteid, and trypsin has also been obtained which did not give the biuret test (Klug, Mays, Schwarzschild). Trypsin dissolves in water and glycerine, while Kühne's trypsin was insoluble in glycerine. It is very sensitive to heat, and even the body temperature gradually decomposes it (Vernon, Mays). In neutral solution it becomes inactive at 45° C. In dilute soda solution of 3–5 p. m. it is still more readily destroyed (Biernacki, Vernon[5]). The presence of proteid or proteoses has, to a certain extent, a protective action on heating an alkaline trypsin solution, and this has been substantiated by recent investigations of Bayliss and Vernon. The simpler cleavage products have a still greater protective action (Vernon[1]). Trypsinogen, according to the unanimous statements of several experimenters, is more resistant towards alkalies than trypsin. Trypsin is gradually destroyed by gastric juice and even by digestive hydrochloric acid alone.

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[1] Compt. rend., **138**.

[2] Gaz. hebdomadaire, 1857, Nos. **15**, **16**, **19**, cited from Bunge, Lehrbuch, **4. Aufl.**, 185.

[3] See Maly's Jahresber., 8, 254.

[4] In this connection see Vines, Annals of Botany, **16**, **17**, **18**, **19**, and **Oppenheimer**, Die Fermente, 1900.

[5] Kühne, Verh. d. naturh.-med. Vereins zu Heidelberg (N. F.), **1**, 3; Klug, Math naturw. Ber. aus Ungarn, 18, 1902; Levene, Amer. Journ. of Physiol., **5**; Mays, Zeitschr. f. physiol. Chem., **38**; Vernon, Journ. of Physiol., **28** and **29**; Biernacki. Zeitschr. f. Biologie, **28**; Schwarzschild, Hofmeister's Beiträge, **4**,.

The preparation of pure trypsin has been tried by various experimenters. The most careful work in this direction was done by **Kühne** and **Mays**. Various methods have been suggested by **Mays**, but we cannot enter into a discussion of them. A very pure preparation can be obtained by making use of the combined salting out with NaCl and $MgSO_4$. A very active solution, and one that can be kept for a long time (for more than twenty years according to **Hammarsten**), can be obtained by extracting with glycerine (**Heidenhain**[2]). An impure but still very active infusion can be obtained after a few days by allowing the finely divided gland to stand with water which contains 5–10 c.c. chloroform per litre (**Salkowski**) at the temperature of the room. This infusion can be kept very active for several years at the cellar temperature. For digestion experiments the active commercial trypsin preparations can be employed.

Like other enzymes, trypsin is characterized by its action, and this action consists in dissolving protein and in splitting it into simpler products, mono- and diamino-acids, tryptophane, etc., in alkaline, neutral, and indeed in very faintly acid solutions. This action has been so far considered as characteristic for trypsin. Recent investigations seem to indicate that this action is not due to one enzyme alone but to the combined action of several enzymes.

There is no question that in the pancreas there occurs besides trypsin also an enzyme similar to erepsin (**Bayliss** and **Starling**, **Vernon**[3]). According to the latter this erepsin has a strong action upon peptone, and he believes that the peptone-splitting action of a pancreas infusion is in great part due to the erepsin. The pancreas besides these also contains a nuclease (see page 380), whose relationship to pancreas erepsin has not been determined.

The unity of trypsin has also been disputed from another point of view. According to **Pollak** the trypsin (in the ordinary sense) contains a second enzyme, which does not act upon protein but only upon gelatine, and he calls this enzyme *glutinase*. This glutinase is much more resistant towards acids than trypsin, and by proper treatment with acids **Pollak**[4] was able to change a pancreas infusion so that it acted upon gelatine and not upon certain proteins. The correctness of these statements has, indeed, not been generally accepted; nevertheless, we have here a warning to be careful as to the conclusions drawn from results where impure infusions are used. For many experiments it is undoubtedly advisable to use the natural pancreatic juice.

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[1] Bayliss, Arch. des scienc. biolog. de St. Pétersbourg, **11**, Suppl.; Vernon, Journ. of Physiol., **31**.

[2] Pflüger's Arch., **10**.

[3] Bayliss and Starling, Journ. of Physiol., **30**; Vernon, *ibid.*, **30**.

[4] Hofmeister's Beiträge, **6**. Contradictory statements may be found in Ehrenreich, cited in Biochem. Centralbl., **4**.

As in recent times the unity of trypsin has been in doubt, the following statements apply only to the enzyme which we have been in the habit of calling trypsin.

The *action of trypsin on proteins* is best demonstrated by the use of fibrin. Very considerable quantities of this protein body are dissolved by a small amount of trypsin at 37–40° C. It is always necessary to make a control test with fibrin alone, with or without the addition of alkali. Fibrin is dissolved by trypsin without any putrefaction; the liquid has a pleasant odor somewhat like bouillon. To completely exclude putrefaction a little thymol, chloroform, or toluene should be added to the liquid. Tryptic digestion differs essentially from pepsin digestion, irrespective of the difference in the digestive products, in that the first takes place in neutral or alkaline reaction and not, as is necessary for peptic digestion, in an acidity of 1–2 p. m. HCl, and further by the fact that the proteins dissolve in trypsin digestion without previously swelling up.

As trypsin not only dissolves proteids, but also other protein substances such as gelatine, this latter body may be used in detecting trypsin. The liquefaction of strongly disinfected gelatine is, according to FERMI,[1] a very delicate test for trypsin or tryptic enzymes. Various suggestions for the use of gelatine in the trypsin test have been made, but in consideration of the above statements of POLLAK in regard to glutinase it is probably best for the present to discard the use of gelatine in detecting trypsin.

For the quantitative estimation of trypsin by measuring the rapidity of digestion we generally make use of the method of METT, described under pepsin digestion. Another method, suggested by WEISS, consists in determining the nitrogen in the filtrate after coagulation with heat and acetic acid. LÖHLEIN recommends the titration method of VOLHARD as used in pepsin determinations, and has given directions for its use.[2]

Many circumstances exert a marked influence on the *rapidity of the trypsin digestion*. With an increase in the *quantity of enzyme* present the digestion is hastened, at least to a certain point. According to PAWLOW and his school, the rule of SCHÜTZ-BORISSOW is perfectly applicable to trypsin, and the amount digested is proportional to the square root of the quantity of ferment. Based upon the investigations of BAYLISS, HEDIN, and LÖHLEIN,[3] this assumption does not seem to have sufficient foundation, and further experiments in this direction are very desirable, as so far experimenters have worked with pancreas infusions or commercial trypsin preparations, which are generally impure mixtures of enzymes. Tryptic digestion is also accelerated by an increase of *temperature*, at least to about 40° C., at which temperature the protein is very rapidly dissolved by the trypsin. The *reaction* is also of the greatest importance. Trypsin acts energetically in neutral, or still better in alkaline, solutions, and best in an alkalinity of 3–4 p. m. Na_2CO_3; but the nature of the protein is also of importance. The action of the alkali depends upon the number of hydroxyl ions (DIETZE, KANITZ), and according to KANITZ[1] the digestion proceeds best in those solutions which are 1/70–1/200 normal in regard to hydroxyl ions. Free mineral acids, even in very small quantities, completely prevent the digestion. If the acid is not actually free, but combined with protein bodies, then the digestion may take place quickly when the acid combination is not in too great excess (CHITTENDEN and CUMMINS). Organic acids act less disturbingly, and in the presence of 0.2 p. m. lactic acid and the simultaneous presence of bile and common salt the digestion may indeed proceed more quickly than in a faintly alkaline liquid (LINDBERGER). The statement of RACHFORD and SOUTHGATE, that the bile can prevent the injurious action of the hydrochloric acid, and that a mixture of pancreatic juice, bile, and hydrochloric acid digests better than a neutral pancreatic juice, could not be substantiated by CHITTENDEN and ALBRO. That bile has an action tending to aid the tryptic digestion has been shown by many investigators and recently by BRUNO, ZUNTZ and USSOW.[2]

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[1] Arch. f. Hygiene, **12** and **55**.

[2] Weiss, Zeitschr. f. physiol. Chem., **40**; Löhlein, Hofmeister's Beiträge, **7**.

[3] Pawlow, Die Arbeit der Verdauungsdrüsen, Wiesbaden, 1898, p. 33; Bayliss, Arch. des scienc. biolog. de St. Pétersbourg, **11**, Suppl.; Hedin, Journ. of Physiol., **32**; Löhlein, l. c.

Carbon dioxide, according to SCHIERBECK,[3] has a retarding action in acid solutions, but it accelerates the tryptic digestion in faintly alkaline liquids. *Foreign bodies*, such as borax and potassium cyanide, may promote tryptic digestion, while other bodies, such as salts of mercury, iron, and others (CHITTENDEN and CUMMINS), or salicylic acid in large quantities, may have a preventive action. According to WEISS[4] the halogen alkali salts disturb tryptic digestion only slightly, and NaCl seems to have the strongest action. The sulphates have a much stronger retarding action than the chlorides. Borax had no influence, while sodium phosphate, on the contrary, had a strong accelerating action. The *nature of the proteins* is also of importance. Unboiled fibrin is, relatively to most other proteins, dissolved so very quickly that the digestion test with raw fibrin gives an incorrect idea of the power of trypsin to dissolve coagulated protein bodies in general. Boiled fibrin is digested with much greater difficulty and requires also a higher alkalinity: 8 p. m. Na_2CO_3 (VERNON[1]). The resistance of certain native protein solutions, such as blood-serum and egg-white, against the action of trypsin is remarkable; a behavior which can be explained by the occurrence of antitrypsin in these solutions. An *accumulation of the products of digestion* tends to hinder the trypsin digestion.

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[1] Kanitz, Zeitschr. f. physiol. Chem., **37**, who also cites Dietze.

[2] Chittenden and Cummins, Studies from the Physiol. Chem. Laboratory of Yale College, New Haven, 1885, **1**, 100; Lindberger, Maly's Jahresber., **13**; Rachford and Southgate, Medical Record, 1895; Chittenden and Albro, Amer. Journ. of Physiol., **1**, 1898; Rachford, Journ. of Physiol., **25**; Bruno, l. c.; Zuntz and Ussow, Arch. f. (Anat. u.) Physiol., 1900.

[3] Skand. Arch. f. Physiol., **3**.

[4] l. c.

The Products of the Tryptic Digestion. In the digestion of unboiled fibrin a globulin which coagulates at 55–60° C. may be obtained as an intermediate product (HERRMANN[2]). Besides this one obtains from fibrin, as well as from other proteins, the products previously mentioned in Chapter II. In trypsin digestion the cleavage may proceed so far that the mixture fails to give the biuret reaction. This does not indicate, as E. FISCHER and ABDERHALDEN have shown, a complete cleavage of the protein molecule into mono- and diamino-acids, etc. In tryptic digestion, as shown by ABDERHALDEN and REINBOLD,[3] using the protein edestin, a gradual cleavage of the protein takes place, and thereby certain amino-acids, like tyrosine and tryptophane, are readily and completely split off, while others, like leucine, alanine, aspartic, acid, and glutamic acid, are slowly and less readily split off, and others, such as α-proline, phenylalanine, and glycocoll, stubbornly resist the cleavage action of the trypsin. The polypeptide-like bodies discovered by FISCHER and ABDERHALDEN, which are produced in digestion, and which do not give the biuret reaction, are the atomic complexes which resist the action of trypsin. These polypeptides contain the pyrrolidine carboxylic acid and phenylalanine groups of the protein, but also yield other monamino-acids such as leucine, alanine, glutamic acid, and aspartic acid. In tryptic digestion no more nitrogen is split off as ammonia than on hydrolysis with acids (MOCHIZUKI), which is a difference between trypsin and the autolytic enzymes. Among the above-mentioned products we find on the autodigestion of the gland other substances, such as oxyphenylethylamine (EMERSON), which is produced from tyrosine by fermentive CO_2 cleavage; also uracil (LEVENE), guanidine (KUTSCHER and OTORI), the purine bases, which originate from the nuclein bodies, and choline, which latter is formed from lecithin (KUTSCHER and LOHMANN[4]). If putrefaction is not completely prevented, still other bodies occur which will be considered later in connection with the putrefactive processes in the intestine.

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[1] Journ. of Physiol., 28.

[2] Herrmann, Zeitschr. f. physiol. Chem., 11.

[3] Zeitschr. f. physiol. Chem., 44 and 46. See also Chapter II.

[4] Fischer and Abderhalden, Zeitschr. f. physiol. Chem., 39; Mochizuki, Hofmeister's Beiträge, 1; Emerson, *ibid.*, 1; Levene, Zeitschr. f. physiol. Chem., 37; Kutscher and Lohmann, *ibid.*, 39; Kutscher and Otori, *ibid.*, 43, and Centralbl. f. Physiol., 18.

The Action of Trypsin upon other Bodies. The *nucleoproteids* and *nucleins* are so digested that the proteid complex is separated from the nucleic acid and then digested. The nucleic acids may, nevertheless, be somewhat changed (ARAKI), which is probably brought about by another enzyme, the *nuclease* (SACHS). A cleavage of nucleic acids with the setting free of phosphoric acid and purine bases is, according to IWANOFF,[1] not brought about by trypsin. This splitting is first produced by the action of nuclease or erepsin (see page 380). *Gelatine* is dissolved and digested by pancreatic juice. A cleavage with the separation of glycocoll and leucine does not occur (KÜHNE and EWALD), or only to a trivial extent (REICH-HERZBERGE[2]).

The *gelatine-forming substance* of the connective tissues is not directly dissolved by trypsin, but only after it has been treated with acids or soaked in water at 70° C. By the action of trypsin on hyaline *cartilage* the cells dissolve, leaving the nucleus. The matrix is softened and shows an indistinctly constructed network of collagenous substance (KÜHNE and EWALD). The *elastic substance*, the *structureless membranes*, and the *membrane of the fat-cells*, are also dissolved. *Parenchymatous organs*, such as the liver and the muscles, are dissolved all but the nuclei, connective tissue, fat-corpuscles, and the remainder of the nervous tissue. If the muscles are boiled, then the connective tissue is also dissolved. *Mucin* is dissolved and split by trypsin, while *chitin* and *horn substance* do not seem to be acted upon by the enzyme. *Oxyhæmoglobin* is decomposed by trypsin with the splitting off of hæmatin. Trypsin has no action upon fats and carbohydrates.

We have the investigations of GULEWITSCH, GONNERMANN, SCHWARZSCHILD,[3] E. FISCHER and BERGELL, and ABDERHALDEN[4] upon the action of trypsin on simply constructed substances of known constitution, such as acid amides and several others that give the biuret reaction. An undoubted cleavage on CURTIUS's biuret base was first observed by SCHWARZSCHILD. The investigations of FISCHER and his co-workers are much more complete and important. From these it is shown that the pancreatic juice splits a large number of peptides, as well as di- and tri- or tetrapeptides, while it is without action upon a large number of others. The structure of these plays an important rôle, as, for example, alanyl-glycine, $CH_3.CH(NH_2).CO.NH.CH_2.COOH$, is split, while its isomere glycyl-alanine, $NH_2.CH_2.CO.NH.CH(CH_3).COOH$, is, on the contrary, not split. The nature of the amino-acids existing in the peptide is also of importance.

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[1] Iwanoff, Zeitschr. f. physiol. Chem., **39**, which also contains the literature; Sachs, *ibid.*, **46**.

[2] Kühne and Ewald, Verh. d. naturh.-med. Vereins zu Heidelberg (N. F.), **1**; Reich-Herzberge, Zeitschr. f. physiol. Chem., **34**.

[3] Hofmeister's Beiträge, **4**, where the other works are also cited.

[4] Fischer and Bergell, Ber. d. d. chem. Gesellsch., **36** and **37**; Fischer and Abderhalden, Sitzungsber. der Kgl. Pr. Akad. d. Wissensch., Berlin, 1905.

Those dipeptides which contain alanine as acyl—for example, alanyl-glycine, alanyl-alanine, and alanyl-leucine A—are readily hydrolyzed, while several dipeptides in which α-aminobutyric acid or leucine functionates as acyl are very resistant. The number of amino-acid groups is also of importance, as, for example, triglycyl-glycine is not split, while tetraglycyl-glycine is. In those peptides which are racemic bodies the hydrolysis takes place asymmetrically, so that only one half of the racemic body is attacked, and those active amino-acids result as products which are contained in the natural protein bodies. This hydrolysis of various polypeptides by means of pancreatic juice is of especially great interest from several points of view.

Pancreatic rennin is an enzyme found in the gland and in the juice which coagulates neutral or alkaline milk (KÜHNE and ROBERTS and others). This enzyme is not identical with trypsin, and the optimum of its action lies according to VERNON between 60° and 65°. According to HALLIBURTON and BRODIE[1] casein is converted by the pancreatic juice of the dog into "pancreatic casein," a substance which, in regard to solubility, stands to a certain extent between casein and paracasein (see Chapter XIV), and which is converted into paracasein by rennin. Further investigations on the action of this enzyme upon milk and especially upon pure casein solutions are very desirable

The property of pancreatic juice of giving plastein precipitates is just as inexplicable as in the case of the gastric juice and other enzyme solutions.

Pancreatic Calculi. The concrement from a cystic enlargement of WIRSUNG's duct in a man, as analyzed by BALDONI,[2] contained in 1000 parts as follows: Water 34.4, ash 126.7, protein substances 34.9, free fatty acids 133, neutral fats 124, cholesterin 70.9, soaps and pigment 499.1, parts.

Besides the enzymes which have been discussed in connection with the pancreatic juice, the gland also contains others, among which can be mentioned the enzyme which, according to STOKLASA and his collaborators, occurs chiefly in organs and tissues and which decomposes sugar into alcohol and carbon dioxide, like zymase. According to ŠIMACEK,[3] in the pancreas the glycolysis by means of alcoholic fermentation, and the hydrolysis of the disaccharides, are united together as a specific action, and he has obtained precipitates from cell-free press-fluid with alcohol and ether which brought on both actions without bacterial action. The statements as to the importance of the pancreas for glycolysis are very contradictory, and we therefore refer the reader to what has been previously stated on this subject in Chapter VIII, pages 302 and 303.

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[1] Kühne and Roberts, Maly's Jahresber., **9**; see also Edkins, Journ. of Physiol., **12** (literature references); Halliburton and Brodie, *ibid.*, **20**; Vernon, *ibid.*, **27**.

[2] Maly's Jahresber., **29**, 353.

[3] Stoklasa, see foot-note 1, p. 303; Šimacek, Centralbl. f. Physiol., **17**.

V. The Chemical Processes in the Intestine.

The action which belongs to each digestive secretion may be essentially changed under certain conditions by being mixed with other digestive fluids for various reasons, and also by the action of the enzymes upon each other;[1] and since the digestive fluids which flow into the intestine are mixed with still another fluid, the bile, it will be readily understood that the combined action of all these fluids in the intestine makes the chemical processes going on therein very complicated.

As the acid of the gastric juice acts destructively on ptyalin, this enzyme has no further diastatic action, even after the acid of the gastric juice has been neutralized in the intestine. The bile has, at least in certain animals, a slight diastatic action, which in itself can hardly be of any great importance, but which shows that the bile has not a preventive but rather a beneficial influence on the energetic diastatic action of the pancreatic juice. MARTIN, WILLIAMS, PAWLOW, and BRUNO[2] have observed a beneficial action of the bile on the diastatic action of the pancreas infusion. To this may be added that the organized ferments which occur habitually in the intestine and sometimes in the food have partly a diastatic action and partly produce a lactic-acid and butyric-acid fermentation. The maltose, which is formed from the starch, seems to be converted into dextrose in the intestine. Cane-sugar is inverted in the intestine, and, at least in certain animals, also lactose.[3] There does not seem to be any doubt that cellulose, especially the fine and tender varieties, is in part dissolved in the intestine; still the products formed thereby are not well known. That cellulose undergoes a fermentation in the intestine by the action of micro-organisms, producing marsh-gas, acetic acid, and butyric acid, has been especially shown by TAPPEINER; still it is not known to what extent the cellulose is destroyed in this way.[4] The extensive experiments of ELLENBERGER and his collaborators, and especially the observations of SCHEUNERT upon the digestion of cellulose, are very important. SCHEUNERT[5] finds that the alkaline contents of the cæcum of the horse, pig, and rabbit have the power of dissolving cellulose to a considerable extent. This power increases as the abundance of micro-organisms increases and *vice versa;* but even in the complete absence of these organisms considerable quantities of cellulose are dissolved. The secretion or the extract of the cæcal mucosa or the cæcal glands does not contain a cellulose-dissolving enzyme, and the solution of cellulose in the cæcum seems therefore to be entirely connected with the micro-organisms or their products.

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[1] See Wróblewski and collaborators, Hofmeister's Beiträge, 1.

[2] Martin and Williams, Proceed. of Roy. Soc., 45 and 48; Bruno, foot-note 1, p. 389.

[3] See foot-note 2, p. 379.

[4] On the digestion of cellulose see Henneberg and Stohmann, Zeitschr. f. Biologie, 21, 613; v. Knieriem, *ibid.*, 67; Hofmeister, Arch. f. wiss. u. prakt. Thierheilkunde, 11; Weiske, Zeitschr. f. Biologie, 22, 373; Tappeiner, *ibid.*, 20 and 24; Mallèvre, Pflüger's Arch., 49; Omeliansky, Arch. d. scienc. biol. de St. Pétersbourg, 7; E. Müller, Pflüger's Arch., 83; Lohrisch, Zeitschr. f. physiol. Chem., 47 (literature).

[5] Ellenberger, Arch. f. (Anat. u.) Physiol., 1906; Scheunert, Zeitschr. f. physiol. Chem., 48.

The bile has, as shown by MOORE and ROCKWOOD [1] and then especially by PFLÜGER, the property to a high degree of dissolving fatty acids, especially oleic acid, which itself is a solvent for other fatty acids, and hence, as will be seen later, it is of great importance in the absorption of fat. It is also of great importance that the bile, as previously stated, not only activates the steapsinogen, but that, as first shown by NENCKI and RACHFORD,[2] it accelerates the fat-splitting action of the steapsin. According to v. FÜRTH and SCHÜTZ [3] the bile-salts are the active constituents of the bile in this cleavage, and the fatty acids set free can combine with the alkalies of the intestinal and pancreatic juices and the bile, producing soaps which are of great importance in the emulsification of the fats.

If to a soda solution of about 1–3 p. m. Na_2CO_3 is added pure, perfectly neutral olive-oil in not too large quantity, a transient emulsion is obtained after vigorous shaking. If, on the contrary, one adds to the same quantity of soda solution an equal amount of commercial olive-oil (which always contains free fatty acids), the vessel need only be turned over for the two liquids to mix, and immediately there appears a very finely divided and permanent emulsion, making the liquid appear like milk. The free fatty acids of the commercial oil, which is always somewhat rancid, combine with the alkali to form soaps which act to emulsify the fats (BRÜCKE, GAD, LOEWENTHAL [4]). This emulsifying action of the fatty acids split off by the pancreatic juice is undoubtedly assisted by the habitual occurrence of free fatty acids in the food, as well as by the splitting off of fatty acids from the neutral fats in the stomach (see page 363).

Bile completely prevents peptic zymolysis in artificial digestion, because it retards the swelling up of the proteins. The passage of bile into the stomach during digestion, on the contrary, seems, according to several investigators, especially ODDI and DASTRE,[5] to have no disturbing action on gastric digestion. According to BOLDIREFF,[6] in continuous starvation, on feeding fat and food rich in fat, as well as after large amounts of acid, a mixture of bile, pancreatic juice, and intestinal juice passes readily into the stomach. After food rich in fat, which retards the secretion of gastric juice and the motility of the stomach, a digestion due to this alkaline mixture may take place in the stomach.

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[1] Proceedings of Roy. Soc., **60**, and Journ. of Physiol., **21**. In regard to Pflüger's work see Absorption.

[2] Nencki, Arch. f. exp. Path. u. Pharm., **20**; Rachford, Journal of Physiol., **12**.

[3] Centralbl. f. Physiol., **20**.

[4] Brücke, Wien. Sitzungsber., **61**, Abt. 2; Gad, Arch. f. (Anat. u.) Physiol., 1878; Loewenthal, *ibid.*, 1897.

[5] Oddi, in Centralbl. f. Physiol., **1**, 312; Dastre, Arch. de Physiol. (5), **2**, 316.

[6] Centralbl. f. Physiol., **18**, 457.

Bile itself has no solvent action on proteins in neutral or alkaline reaction, but still it may exert an influence on protein digestion in the intestine. The acid contents of the stomach, containing an abundance of proteins, give with the bile a precipitate of proteins and bile-acids. This precipitate carries a part of the pepsin with it, and for this reason, and also on account of the partial or complete neutralization of the acid of the gastric juice by the alkali of the bile and the pancreatic juice, the pepsin digestion cannot proceed further in the intestine. On the contrary, the bile does not disturb the digestion of proteins by the pancreatic juice in the intestine. The action of these digestive secretions, as above stated, is not disturbed by the bile, not even by the faintly acid reaction due to organic acids; but, on the contrary, the action of trypsin is accelerated by the bile. In a dog killed while digestion is going on, the faintly acid, bile-containing material of the intestine shows regularly a strong digestive action on proteins.

The precipitate formed on the meeting of the acid contents of the stomach with the bile easily redissolves in an excess of bile and also in the NaCl formed in the neutralization of the hydrochloric acid of the gastric juice. This may take place even under faintly acid reaction. Since in man the excretory ducts of the bile and the pancreatic juice open near one another, in consequence of which the acid contents of the stomach are probably immediately in great part neutralized by the bile as soon as it enters, it is doubtful whether a precipitation of proteins by the bile occurs in the intestine.

Besides the previously mentioned processes caused by enzymes, there are others of a different nature going on in the intestine, namely, the fermentation and putrefaction processes caused by micro-organisms. These are less intense in the upper parts of the intestine, but increase in intensity towards the lower part of the same, and decrease in the large intestine because of the consumption of fermentable material and by the removal of water by absorption. Fermentation processes, but only very slight putrefaction, occur in the small intestine of man. MACFADYEN, M. NENCKI, and N. SIEBER[1] have investigated a case of human anus præternaturalis, in which the fistula occurred at the lower end of the ileum, and they were able to investigate the contents of the intestine after it had been exposed to the action of the mucous membrane of the entire small intestine. The mass was yellow or yellowish brown, due to bilirubin, and had an acid reaction which, on a mixed but chiefly animal diet, calculated as acetic acid, amounted to 1 p. m. The contents were nearly odorless, having an empyreumatic odor recalling that of volatile fatty acids, and only seldom had a putrid odor resembling that of indol. The essential acid present was acetic acid, accompanied by fermentation lactic acid and paralactic acid, volatile fatty acids, succinic acid, and bile-acids. Coagulable proteins, peptone, mucin, dextrin, dextrose, and alcohol were present. Leucine and tyrosine could not be detected.

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[1] Arch. f. exp. Path. u. Pharm., 28.

According to the above-mentioned investigators, the proteins are only to a very slight extent, if at all, decomposed by the microbes in the small intestine of man. The organisms present in the small intestine preferably decompose the carbohydrates, forming ethyl alcohol and the above-mentioned organic acids.

Further investigations of JAKOWSKY and of AD. SCHMIDT [1] led to the same result, namely, that in man the putrefaction of the proteins takes place chiefly in the large intestine, and the conditions are the same in carnivora. In these latter it has been possible to follow the intestinal digestion by investigating the contents of the various parts of the intestine as well as by forming fistulas. LONDON and SULIMA produced fistulas in different dogs in the duodenum, jejunum, and ileum, and could follow the digestion of boiled egg-white. A complete destruction of the same took place, so that 99.7 per cent of the protein was dissolved and flowed out of the fistula at the ileum (2–3 cm. in front of the cæcum). The intestinal contents gave the biuret reaction very faintly, and the dissolved substance seemed to have been transformed into end-products. MAETZKE,[2] who carried on his investigations on a dog with a fistula at the lower end of the ileum, on feeding meat never found a putrid or fæcal odor to the intestinal contents. The digestion and absorption of the meat as well as of the carbohydrate was also nearly complete. Leucine and tyrosine were looked for but not found, and the absence of these bodies was explained by the fact that they were absorbed.

Because of the absorption it is also difficult to state the extent of destruction of the proteins in the intestine. Several experimenters who have investigated the intestinal contents of dogs during the digestion of meat have detected amino-acids such as leucine, tyrosine, lysine, and arginine (KUTSCHER and SEEMANN), glutamic and aspartic acids, alanine (LONDON), and polypeptides not giving the biuret reaction (ABDERHALDEN [3]).

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[1] Jakowsky, Arch. des scienc. biol. de St. Pétersbourg, **1**; Ad. Schmidt, Arch. f. Verdauungskr., **4**.

[2] London and Sulima, Zeitschr. f. physiol. Chem., **46**; Maetzke, Beobachtungen an Hunden mit Anus præternaturalis, Inaug.-Dissert. Breslau, 1905.

[3] Kutscher and Seemann, Zeitschr. f. physiol. Chem., **34**; Abderhalden, *ibid.*, **44**; London, *ibid.*, **47**.

The digestion and absorption of proteins in the stomach and small intestine may be nearly complete, but this is not always so. In experiments with raw egg-white London and Sulima reobtained about 73 per cent of the coagulable protein from the ileum fistula, and in the entire intestine from the pylorus to the cæcum only about 12 per cent of the food substance was absorbed. Also in milk-feeding a considerable part of the protein passes into the large intestine (Berlatzki[1]).

As above remarked, ordinarily no putrefaction takes place in the small intestine but occurs generally only in the large intestine. This putrefaction of the proteins is not the same as the pancreatic digestion. In putrefaction the decomposition goes much further and a mixture of products is obtained which have become known through the labors of numerous investigators, especially Nencki, Baumann, Brieger, H. and E. Salkowski, and their pupils. The products which are formed in the putrefaction of proteins are (in addition to *proteoses, peptones, amino-acids,* and *ammonia*) *indol, skatol, paracresol, phenol, phenylpropionic acid,* and *phenylacetic acid,* also *paraoxyphenylacetic acid* and *hydroparacumaric acid* (besides paracresol, produced in the putrefaction of tyrosine), *volatile fatty acids, carbon dioxide, hydrogen, marsh-gas, methylmercaptan,* and *sulphuretted hydrogen.* In the putrefaction of gelatine neither tyrosine nor indol is formed, while *glycocoll* is produced instead.

Among these products of decomposition a few are of special interest because of their behavior within the organism and because after their absorption they pass into the urine. A few, such as the oxyacids, pass unchanged into the urine. Others, such as phenols, are directly transformed into ethereal sulphuric acids by synthesis, and are eliminated as such by the urine; on the contrary, others, such as indol and skatol, are only converted into ethereal sulphuric acids after oxidation (for details see Chapter XV). The quantity of these bodies in the urine varies also with the extent of the putrefactive processes in the intestine; at least this is true for the ethereal sulphuric acids. Their quantity increases in the urine with a stronger putrefaction, and the reverse takes place, namely, a disappearance from the urine, or a great reduction in quantity, as Baumann, Harley and Goodbody[2] have shown by experiments on dogs, when the intestine was disinfected by various agents.

Among the above-mentioned putrefactive products in the intestine the two following, indol and skatol, should be especially noted.

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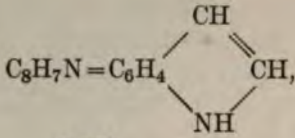
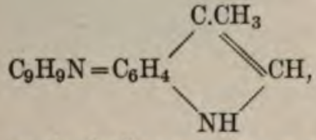
[1] See Biochem. Centralbl., 2.

[2] Baumann, Zeitschr. f. physiol. Chem., 10; Harley and Goodbody, Brit. Med. Journ., 1899.

Indol,  $C_8H_7N=C_6H_4\left\langle\begin{array}{c}CH\\ \\NH\end{array}\right\rangle CH$, and **Skatol,** or METHYL-INDOL,  $C_9H_9N=C_6H_4\left\langle\begin{array}{c}C.CH_3\\ \\NH\end{array}\right\rangle CH$, are two bodies which stand in close relationship to the indigo substances and are formed in variable quantities from protein compounds under different conditions. Hence they occur habitually in the human intestinal canal, and, after oxidation into indoxyl and skatoxyl respectively, pass, at least partly, into the urine as the corresponding ethereal sulphuric acids and also as glucuronic acids.

These two bodies have been prepared synthetically in many ways. Both may be obtained from indigo by reducing it with tin and hydrochloric acid and heating this reduction product with zinc-dust (BAEYER [1]). Indol may be formed from skatol by passing it through a red-hot tube. Indol suspended in water is in part oxidized into indigo-blue by ozone (NENCKI [2]).

Indol and skatol crystallize in shining leaves, and their melting-points are 52° and 95° C. respectively. Indol has a peculiar excrementitious odor, while skatol has an intense fetid odor (skatol obtained from indigo is odorless). Both bodies are easily volatilized by steam, skatol more easily than indol. They may both be removed from the watery distillate by ether. Skatol is the more insoluble of the two in boiling water. Both are easily soluble in alcohol and give with picric acid a compound crystallizing in red needles. If a mixture of the two picrates be distilled with ammonia, they both pass over without decomposition; while if they are distilled with caustic soda, the indol but not the skatol is decomposed. The watery solution of indol gives with fuming nitric acid a red liquid and then a red precipitate of nitroso-indol nitrate (NENCKI). It is better first to add two or three drops of nitric acid and then a 2 per cent solution of potassium nitrite, drop by drop (SALKOWSKI [3]). Skatol does not give this reaction. An alcoholic solution of indol treated with hydrochloric acid colors a pine chip cherry-red. Skatol does not give this reaction. Indol gives a deep reddish-violet color with sodium nitroprusside and alkali (LEGAL'S reaction). On acidifying with hydrochloric acid or acetic acid the color becomes pure blue. Skatol does not act the same. The alkaline solution is yellow and becomes violet on acidifying with acetic acid and boiling. Skatol dissolves in concentrated hydrochloric acid with a violet coloration. On warming skatol with sulphuric acid a beautiful purple-red coloration is obtained (CIAMICIAN and MAGNANINI [1]).

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[1] Annal. d. Chem. u. Pharm., **140**, and Suppl., **7**, 56; also Ber. d. deutsch. chem. Gesellsch., **1**.

[2] Ber. d. deutsch. chem. Gesellsch., 8, 727, and *ibid.*, 722 and 1517.

[3] Zeitschr. f. physiol. Chem., 8, 447. In regard to newer reactions for indol and skatol, see Steensma, *ibid.*, **47**.

For the detection of indol and skatol in, and their preparation from, excrement and putrefying mixtures, the main points of the usual method are as follows: The mixture is distilled after acidifying with acetic acid; the distillate is then treated with alkali (to combine with any phenols which may be present) and again distilled. From this second distillate the two bodies, after the addition of hydrochloric acid, are precipitated by picric acid. The picrate precipitate is then distilled with ammonia. The two bodies are obtained from the distillate by repeated shaking with ether and evaporation of the several ethereal extracts. The residue, containing indol and skatol, is dissolved in a very small quantity of absolute alcohol and treated with 8–10 vols. of water. Skatol is precipitated, but not the indol. The further treatment necessary for their separation and purification will be found in other works.[2]

The *gases* which are produced by the decomposition processes are mixed in the intestinal tract with the atmospheric air swallowed with the saliva and food, and as the gas developed in the decomposition of different foods varies, so the mixture of gases after various foods should have a dissimilar composition. This is found to be true. *Oxygen* is found only in very faint traces in the intestine; this may be accounted for in part by the formation of reducing substances in the fermentation processes which combine with the oxygen, and partly, perhaps chiefly, to a diffusion of the oxygen through the tissues of the walls of the intestine. To show that these processes take place mainly in the stomach the reader is referred to page 372, on the composition of the gases of the stomach. *Nitrogen* is habitually found in the intestine, and it is probably due chiefly to the swallowed air. The *carbon dioxide* originates partly from the contents of the stomach, partly from the putrefaction of the proteins, partly from the lactic-acid and butyric-acid fermentation of carbohydrates, and partly from the setting free of carbon dioxide from the alkali carbonates of the pancreatic and intestinal juices by their neutralization through the hydrochloric acid of the gastric juice and by organic acids formed in the fermentation. *Hydrogen* occurs in largest quantities after a milk diet, and in smallest quantities after a purely meat diet. This gas seems to be formed chiefly in the butyric-acid fermentation of carbohydrates, although it may occur in large quantities in the putrefaction of proteins under certain circumstances.

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[1] Ber. d. d. chem. Gesellsch., **21**, 1928.

[2] For quantitative, colorimetric determinations of indol in fæces, see Einhorn and Huebner, Salkowski's Festschrift, Berlin, 1904.

There is no doubt that the *methylmercaptan* and *sulphuretted hydrogen* which occur normally in the intestine originate from the proteins. The *marsh-gas* undoubtedly originates in the putrefaction of proteins. As proof of this RUGE[1] found 26.45 per cent marsh-gas in the human intestine after a meat diet. He found a still greater quantity of this gas after a vegetable (leguminous) diet; this coincides with the observation that marsh-gas may be produced by a fermentation of carbohydrates, but especially of cellulose (TAPPEINER[2]). Such an origin of marsh-gas, especially in herbivora, is to be expected. A small part of the marsh-gas and carbon dioxide may also arise from the decomposition of lecithin (HASEBROEK[3]).

Putrefaction in the intestine not only depends upon the composition of the food, but also upon the albuminous secretions and the bile. Among the constituents of bile which are changed or decomposed there are not only the pigments—the bilirubin yields urobilin and a brown pigment—but also the bile-acids, especially taurocholic acid. Glycocholic acid is more stable, and a part is found unchanged in the excrement of certain animals, while taurocholic acid is so completely decomposed that it is entirely absent in the fæces. In the fœtus, on the contrary, in whose intestinal tract no putrefaction processes occur, undecomposed bile-acids and bile-pigments are found in the contents of the intestine. The transformation of bilirubin into urobilin does not occur, as previously stated, in man in the small but in the large intestine.

As under normal conditions no putrefaction, or at least none worth mentioning, occurs in the small intestine, and as often nearly all the protein of the food is absorbed, it follows that ordinarily it is the secretions and cells rich in protein which undergo putrefaction. That the secretions rich in proteins are destroyed in putrefaction in the intestine follows from the fact that putrefaction may also continue during complete fasting. From the observations of MÜLLER[4] upon CETTI it was found that the elimination of indican during starvation rapidly decreased and after the third day of starvation it had entirely disappeared, while the phenol elimination, which at first decreased so that it was nearly minimum, increased again from the fifth day of starvation, and on the eight or ninth day it was three to seven times as much as in man under ordinary circumstances. In dogs, on the contrary, the elimination of indican during starvation is considerable, but the phenol elimination is slight. Among the secretions which undergo putrefaction in the intestine, the pancreatic juice, which putrefies most readily, takes first place.

From the foregoing facts it must be concluded that the products formed by the putrefaction in the intestine are in part the same as those formed in digestion. The putrefaction may be of benefit to the organism in so far as the formation of such products as proteoses, peptones, and perhaps also certain amino-acids is concerned. The question has indeed been asked (PASTEUR), is digestion possible without micro-organisms? NUTTAL and THIERFELDER have shown that guinea-pigs removed from the uterus of the mother by Cæsarian section could with sterile air digest well and assimilate sterile food (milk or crackers) in the complete absence of bacteria in the intestine, and developed normally and increased in weight. SCHOTTELIUS[1] has arrived at other results by experiments with hens. The chickens, hatched under sterile conditions, kept in sterile rooms and fed with sterile food, had continuous hunger and ate abundantly, but soon died, in about the same time as a starving chicken. On mixing with the food of other chickens, at the proper time, a variety of bacteria from hen fæces, they gained weight again and recovered.

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[1] Wien. Sitzungsber., **44**.

[2] Zeitsch. f. Biologie, **20** and **24**.

[3] Zeitschr. f. physiol. Chem., **12**.

[4] Berlin. klin. Wochenschr., 1887.

The bacterial action in the intestinal canal is, at least in certain cases, necessary, and it acts in the interest of the organism. This action may, by the formation of further cleavage products, involve a loss of valuable material to the organism, and it is therefore important that putrefaction in the intestine be kept within certain limits. If an animal is killed while digestion in the intestine is going on, the contents of the small intestine give out a peculiar but not putrescent odor. Also the odor of the contents of the large intestine is far less offensive than a putrefying pancreas infusion or a putrefying mixture rich in protein. From this one may conclude that putrefaction in the intestine is ordinarily not nearly so intense as outside of the organism.

It seems thus to be provided, under physiological conditions, that putrefaction shall not proceed too far, and the factors which here come under consideration are probably of different kinds. Absorption is undoubtedly one of the most important of them, and it has been proved by actual observation that the putrefaction increases, as a rule, as the absorption is checked and fluid masses accumulate in the intestine. The character of the food also has an unmistakable influence, and it seems as if a large quantity of carbohydrates in the food acts against putrefaction (HIRSCHLER[2]). It has been shown by PÖHL, BIERNACKI, ROVIGHI, WINTERNITZ, SCHMITZ, and others[3] that milk and kephir have a specially strong preventive action on putrefaction. This action is not due to the casein, but chiefly to the lactose and also in part to the lactic acid.

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[1] Nuttal and Thierfelder, Zeitschr. f. physiol. Chem., **21** and **22**; Schottelius, Arch f. Hygiene, **34** and **42**.

[2] Hirschler, Zeitschr. f. physiol. Chem., **10**; Zimnitzki, *ibid.*, **39** (literature).

[3] Schmitz, *ibid.*, **17**, 401, which gives references to the older literature, and **19**. See also Salkowski, Centralbl. f. d. med. Wiss., 1893, 467, and Seelig, Virchow's Arch., **146** (literature).

A specially strong preventive action on putrefaction has been ascribed for a long time to the bile. This anti-putrid action does not exist in neutral or faintly alkaline bile, which itself easily putrefies, but to the free bile-acids, especially taurocholic acid (MALY and EMICH, LINDBERGER[1]). There is no question that the free bile-acids have a strong preventive action on putrefaction outside of the organism, and it is therefore difficult to deny such an action in the intestine. Notwithstanding this the anti-putrid action of the bile in the intestine is not considered by certain investigators (VOIT, RÖHMANN, HIRSCHLER and TERRAY, LANDAUER and ROSENBERG[2]) as of great importance.

Biliary fistulas have been established so as to study the importance of the bile in digestion (SCHWANN, BLONDLOT, BIDDER and SCHMIDT,[3] and others). As a result it has been observed that with fatty foods an imperfect absorption of fat regularly takes place and the excrements contain, therefore, an excess of fat and have a light-gray or pale color. The extent of deviation from the normal after the operation is essentially dependent upon the character of the food. If an animal is fed on meat and fat, then the quantity of food must be considerably increased after the operation, otherwise the animal will become very thin, and indeed die with symptoms of starvation. In these cases the excrements have the odor of carrion, and this was considered a proof of the action of the bile in checking putrefaction. The emaciation and the increased want of food depend, naturally, upon the imperfect absorption of the fats, whose high calorific value is reduced and must be replaced by the taking up of larger quantities of other nutritive bodies. If the quantity of proteins and fats be increased, then the latter, which can be only very incompletely absorbed, accumulate in the intestine. This accumulation of the fats in the intestine only renders the action of the digestive juices on proteins more difficult, and thus increases the amount of putrefaction. This explains the appearance of fetid fæces, whose pale color is not due to a lack of bile-pigments, but to a surplus of fat (RÖHMANN, VOIT). If the animal is, on the contrary, fed on meat and carbohydrates, it may remain quite normal, and the leading off of the bile does not cause any increased putrefaction. The carbohydrates may be uninterrupedly absorbed in such large quantities that they replace the fat of the food, and this is the reason why the animal on such a diet does not become emaciated. As with this diet the putrefaction in the intestine is no greater than under normal conditions even though the bile is absent, it would seem that the bile in the intestine exercises no preventive action on putrefaction.

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[1] Maly and Emich, Monatshefte f. Chem., 4; Lindberger, foot-note 2, p. 393.

[2] Voit, Beitr. zur Biologie, Jubiläumschrift, Stuttgart, 1882; Röhmann, Pflüger's Arch., 29; Hirschler and Terray, Maly's Jahresber., 26; Landauer, Math. u. Naturw. Ber. aus Ungarn, 15; Rosenberg, Arch. f. (Anat. u.) Physiol., 1901.

[3] Schwann, Müller's Arch. f. Anat. u. Physiol., 1844; Blondlot, cited from Bidder and Schmidt, Verdauungssäfte, etc., 98.

To this conclusion the objection may be made that the carbohydrates, which are capable of checking putrefaction, can, so to speak, undertake the anti-putrid action of the bile. But as there are also cases (in dogs with biliary fistula) where the intestinal putrefaction is not increased with exclusive meat diet,[1] it is maintained that the absence of bile in the intestine, even by exclusive carbohydrate food, does not always cause an increased putrefaction.

Although the question as to the manner in which the putrefactive processes in the intestine under physiological conditions are kept within certain limits cannot be answered positively, still it may be asserted that the acid reaction of the upper parts of the intestine, and the absorption of water in the lower parts, are important factors.

That the acid reaction in the intestine has a preventive influence on the putrefactive processes follows from the existing relation between the degree of acidity of the gastric juice and the putrefaction in the intestine. After the investigations and observations of KAST, STADELMANN, WASBUTZKI, BIERNACKI and MESTER had proved that an increased putrefaction in the intestine occurred when the quantity of hydrochloric acid in the gastric juice was diminished or deficient, SCHMITZ[2] has lately shown in man that on the administration of hydrochloric acid, producing a hyperacidity of the gastric juice, the putrefaction in the intestine may be checked. The question arises whether the reaction in the small intestine is always acid and whether the acidity is strong enough to prevent putrefaction. In this connection it must be recalled that the acidity of the contents of the small intestine is not due to hydrochloric acid, but chiefly to organic acids, acid salts, and free carbon dioxide. There are several statements as to the reaction of the intestinal contents, although they are somewhat contradictory, by MOORE and ROCKWOOD, MOORE and BERGIN, MATTHES and MARQUARDSEN, I. MUNK, NENCKI and ZALESKI, HEMMETER.[3] From these statements one can conclude that the reaction may vary not only among different animals, but also in the same animals under different conditions. There is no doubt that the acid reaction in many cases is due to the presence of organic acids. On testing with various indicators it has been shown that sometimes the upper parts, and often the lower parts, are acid, due to acid salts such as $NaHCO_3$ and free CO_2, and finally that in certain animals the intestinal contents are alkaline throughout. The question how, under these conditions, putrefaction is excluded, cannot be explained. It is possible, as BIENSTOCK admits, that the explanation lies in an antagonistic bacterial action and that the carbohydrates, especially lactose, which retard putrefaction, form a good nutritive media for those bacteria which destroy the putrefactive producers or retard their development. Perhaps also, according to the experience of CONRADI and KURPJUWEIT,[1] the autotoxines produced by the intestinal bacteria may, by their antiseptic action, keep the putrefactive processes in the intestine within bounds.

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[1] See Hirschler and Terray, l. c.

[2] Zeitschr. f. physiol. Chem., **19**, 401, which includes all the pertinent literature.

[3] Moore and Rockwood, Journ. of Physiol., **21**; Moore and Bergin, Amer. Journ. of Physiol., **3**; Matthes and Marquardsen, Maly's Jahresber., **28**; Munk, Centralbl. f. Physiol., **16**; Nencki and Zaleski, Zeitschr. f. physiol. Chem., **27**; Hemmeter, Pflüger's Arch., **81**.

Excrements. It is evident that the residue which remains after complete digestion and absorption in the intestine must be different, both qualitatively and quantitatively, according to the variety and quantity of the food. In man the quantity of excrement from a mixed diet is 120–150 grams, with 30–37 grams of solids, per twenty-four hours, while the quantity from a vegetable diet, according to VOIT,[2] was 333 grams, with 75 grams of solids. With a strictly meat diet the excrements are scanty, pitch-like, and black. The scanty excrements in starvation have a similar appearance. A large quantity of coarse bread yields a great amount of light-colored excrement. In these cases the fæces are also habitually poorer in nitrogen than after food rich in protein. The individuality also plays an important rôle in the utility of the food and the formation of fæces (SCHIERBECK[3]). If there is a large proportion of fat, it takes a lighter, clay-like appearance. The decomposition products of the bile-pigments seem to play only a small part in the normal color of the fæces.

The constituents of the fæces are of different kinds. In the excrements are found digestible or absorbable constituents of the food, such as muscle fibres, connective tissues, lumps of casein, grains of starch, and fat, which have not had sufficient time to be completely digested or absorbed in the intestinal tract. In addition the excrements contain indigestible bodies, such as the remains of plants, keratin substances, and others; also form-elements originating from the mucous coat and the glands; constituents of the different secretions, such as mucin, cholic acid, dyslysine, and cholesterin (koprosterin or stercorin), purine bases,[4] and enzymes; mineral bodies of the food and the secretions; and, lastly, products of putrefaction or of digestion, such as skatol, indol, volatile fatty acids, purine bases, lime, and magnesia soaps. Occasionally, also, parasites of different kinds occur; and lastly, the excrements contain micro-organisms of various species.

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[1] Bienstock, Arch. f. Hygiene, **39**; Conradi and Kurpjuweit, Münch. med. Wochenschr., **1905**.

[2] Zeitschr. f. Biologie, **25**, 264.

[3] Arch. f. Hygiene, **51**.

[4] In regard to the purine bases in fæces, see Hall, Journ. of Path. and Bacteriol., **9**; Schittenhelm, Arch. f. klin. Med., **81**; Schittenhelm and Krüger, Zeitschr. f. physiol. Chem., **45**.

That the mucous membrane of the intestine by its secretion and by the abundant quantity of detached epithelium contributes essentially to the formation of excrement follows from the discovery first made by L. Hermann and substantiated by others,[1] that a clean, isolated loop of intestine collects material similar to fæces. Human fæces seem to consist in greater part of intestinal secretion and only in a smaller part of residue from food on a meat or milk diet. Many foods produce a large quantity of fæces chiefly by causing an abundant secretion.[2]

The reaction of the excrements is very variable, but in man with a mixed diet it is neutral or faintly alkaline. It is often acid in the inner part, while the outer layers in contact with the mucous coat have an alkaline reaction. In nursing infants it is habitually acid. The odor is perhaps chiefly due to skatol, which was first found in the excrements by Brieger, and so named by him. Indol and other substances also take part in the production of odor. The color is ordinarily light or dark brown, and depends above all upon the nature of the food. Medicinal bodies may give the fæces an abnormal color. The excrements are colored black by bismuth, yellow by rhubarb, and green by calomel. This last-mentioned color was formerly accounted for by the formation of a little mercury sulphide, but now it is said that calomel checks the putrefaction and the decomposition of the bile-pigments, so that a part of the bile-pigments passes into the fæces as biliverdin. In the yolk-yellow or greenish-yellow excrements of nursing infants one can detect bilirubin. Neither bilirubin nor biliverdin seems to exist in the excrements of mature persons under normal conditions. On the contrary, there is found *stercobilin* (Masius and Vanlair), which is identical with urobilin (Jaffé[3]). Bilirubin may occur in pathological cases in the fæces of mature persons. It has been observed in a crystallized state (as hæmatoidin) in the fæces of children as well as of grown persons.

The absence of bile (acholic fæces) causes the excrements to have, as above stated, a gray color, due to large quantities of fat; this may, however, be partly attributed to the absence of bile-pigments. In these cases a large quantity of crystals has been observed which consist chiefly of magnesium soaps or sodium soaps. Hemorrhage in the upper parts of the digestive tract yields, when it is not very abundant, a dark-brown excrement, due to hæmatin.

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[1] Hermann, Pflüger's Arch., 46. See also Ehrenthal, *ibid.*, 48; Berenstein, *ibid.*, 53; Klecki, Centralbl. f. Physiol., 7; 736, and F. Voit, Zeitschr. f. Biologie, 29; v. Moraczewski, Zeitschr. f. physiol. Chem., 25.

[2] In regard to the constitution of fæces with various foods, see Hammerl, Kermauner, Moeller, and Prausnitz, Zeitschr. f. Biologie, 35, and Poda, Micko, Prausnitz and Müller, *ibid.*, 39.

[3] See bile-pigments, Chapter VIII, and urobilin, Chapter XV.

EXCRETIN, so named by MARCET,[1] is a crystalline body occurring in human excrement, but which, according to HOPPE-SEYLER, is perhaps only impure cholesterin (koprosterin or stercorin?). EXCRETOLIC ACID is the name given by MARCET to an oily body with an excrementitious odor.

In consideration of the very variable composition of excrements, their quantitative analyses are of little value and therefore will be omitted.[2]

Meconium is a dark brownish-green, pitchy, mostly acid mass without any strong odor. It contains greenish-colored epithelium cells, cell-detritus, numerous fat-globules, and cholesterin plates. The amount of water is 720–800, and solids 280–200 p. m. Among the solids there are mucin, bile-pigments, and bile-acids, cholesterin, fat, soaps, traces of enzymes, calcium and magnesium phosphates. Sugar and lactic acid, soluble protein bodies and peptones, also leucine and tyrosine and the other products of putrefaction occurring in the intestine, are absent. Meconium may contain undecomposed taurocholic acid, bilirubin and biliverdin, but it does not contain any stercobiline, which is considered as proof of the non-existence of putrefactive processes in the digestive tract of the fœtus.

In medico-legal cases it is sometimes necessary to decide whether spots on linen or other substances are caused by meconium. In such cases the following conditions exist: The spot caused by meconium has a brownish-green color and can be easily separated from the material because, on account of the ropy property of the meconium, it is difficult to wet through. When moistened with water it does not develop any special odor, but on warming with dilute sulphuric acid it smells somewhat fetid. It forms with water a slimy, greenish-yellow liquid containing brown flakes. The solution gives with an excess of acetic acid an insoluble precipitate of mucin; on boiling it does not coagulate. The filtered, watery extract responds to GMELIN'S, but still better to HUPPERT'S reaction for bile-pigments. The liquid precipitated by an excess of milk of lime gives a nearly colorless filtrate, which after concentration shows PETTENKOFER'S reaction.

The contents of the intestine under abnormal conditions are perhaps less the subject of chemical analysis than of an inspection and microscopical investigation or bacteriological examination. On this account the question as to the properties of the contents of the intestine in different diseases cannot be thoroughly treated here.[3]

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[1] Annal. de chim. et de phys., 59.

[2] In regard to these analyses as well as to the faeces under abnormal conditions and to the pertinent literature, see Ad. Schmidt and J. Strassburger, Die Fæces des Menschen, etc., Berlin, 1901 and 1902.

[3] See Schmidt and Strassburger, l. c.

Appendix.

INTESTINAL CONCREMENTS.

Calculi occur very seldom in the human intestine or in the intestine of carnivora, but they are quite common in herbivora. Foreign bodies or undigested residues of food may, when for some reason or other they are retained in the intestine for some time, become incrusted with salts, especially ammonium-magnesium phosphate or magnesium phosphate, and these salts usually form the chief constituent of the concrements. In man they are sometimes oval or round, yellow, yellowish gray, or brownish gray, of variable size, consisting of concentric layers and containing chiefly ammonium-magnesium phosphate and calcium phosphate, besides a small quantity of fat or pigment. The nucleus ordinarily consists of some foreign body, such as the stone of a fruit, a fragment of bone, or something similar. In those countries where bread made from oat-bran is an important food, we often find in the large intestine balls similar to the so-called hair-balls (see below). Such calculi contain calcium and magnesium phosphate (about 70 per cent), oat-bran (15–18 per cent), soaps and fat (about 10 per cent). Concretions which contain very much fat (about 74 per cent) occasionally occur, and those consisting of fibrin clots, sinews, or pieces of meat incrusted with phosphates are also rare.

Intestinal calculi often occur in animals, especially in horses fed on bran. These calculi, which attain a very large size, are hard and heavy (as much as 8 kilos) and consist in great part of concentric layers of ammonium-magnesium phosphate. Another variety of concrements which occurs in horses and cattle consists of gray-colored, often very large, but relatively light stones which contain plant residues and earthy phosphates. Stones of a third variety are sometimes cylindrical, sometimes spherical, smooth, shining, brownish on the surface, consisting of matted hairs and plant-fibres, and termed *hair-balls*. The so-called "ÆGAGROPILÆ," which probably originate from the ANTILOPUS RUPICAPRA, belong to this group, and are generally considered as nothing else than the hair-balls of cattle.

The so-called *oriental bezoar-stone* belongs also to the intestinal concrements, and probably originates from the intestinal tract of the CAPRA ÆGAGRUS and ANTILOPE DORCAS. There may exist two varieties of bezoar-stones. One is olive-green, faintly shining and formed of concentric layers. On heating it melts with the development of an aromatic odor. It contains as chief constituent LITHOFELLIC ACID, $C_{20}H_{36}O_4$, which is related to cholic acid, and besides this a bile-acid, LITHOBILIC ACID. The others are nearly blackish brown or dark green, very glossy, consisting of concentric layers, and do not melt on heating. They contain as chief constituent ellagic acid, a derivative of gallic acid, of the formula $C_{14}H_6O_8$, which, according to GRAEBE,[1] is the dilactone of hexaoxydiphenyldicarboxylic acid and which gives a deep-blue color with an alcoholic solution of ferric chloride. This last-mentioned bezoar-stone originates, to all appearances, from the food of the animal.

Ambergris is generally considered an intestinal concrement of the sperm-whale. Its chief constituent is *ambrain*, which is a non-nitrogenous substance perhaps related to cholesterin. Ambrain is insoluble in water and is not changed by boiling alkalies. It dissolves in alcohol, ether, and oils.

VI. Absorption.

The problem of digestion consists in part in separating the valuable constituents of the food from the useless ones and dissolving or transforming them into forms which are adapted for the processes of absorption. In discussing the absorption processes we must treat of the form into which the different foods are changed before absorption, of the manner in which this is accomplished, and, lastly, of the forces which act in these processes.

Before we can answer the question as to the form in which the proteins are absorbed from the intestinal canal, it is of interest to learn whether the animal body can, perhaps, also utilize such protein as is introduced intravenously, subcutaneously, or into a body-cavity, i.e., evading the intestinal canal, or, as OPPENHEIMER calls it, parenteral.

Since the first investigations of ZUNTZ and v. MERING on this subject several experimenters, such as NEUMEISTER, FRIEDENTHAL and LEWANDOWSKY, MUNK and LEWANDOWSKY, OPPENHEIMER, MENDEL and ROCKWOOD, and others,[2] have shown, without any doubt, that the animal body can more or less completely utilize different, parenterally introduced proteins, although different varieties of animals show a difference in this regard. Still we do not know where and how these foreign proteins are changed and assimilated.

If the animal body can assimilate parenterally introduced protein, then the question arises, whether it can also take up undigested protein from the intestinal canal and utilize it. In this regard we have the observations of a large number of investigators, such as BRÜCKE, BAUER and VOIT, EICH-

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[1] Ber. d. d. chem. Gesellsch., **36**.

[2] Zuntz and v. Mering, Pflüger's Arch., **32**; Neumeister, Verh. d. phys.-med. Gesellsch. zu Würzburg, 1889, and Zeitschr. f. Biologie, **27**; Friedenthal and Lewandowsky, Arch. f. (Anat. u.) Physiol., 1899; Munk and Lewandowsky, *ibid.*, 1899, Suppl.; Oppenheimer, Hofmeister's Beiträge, **4**; Mendel and Rockwood, Amer. Journ. of Physiol., **12**.

HORST, CZERNY and LATSCHENBERGER, VOIT and FRIEDLÄNDER,[1] who have shown that non-peptonized protein can be absorbed from the intestine. In the experiments of the two last-mentioned investigators neither casein (as milk) nor hydrochloric-acid myosin or acid albuminate (in acid solution) was absorbed, while, on the contrary, about 21 per cent of ovalbumin or seralbumin and 69 per cent of alkali albuminate (dissolved in alkali) were absorbed. MENDEL and ROCKWOOD, on the contrary, in experiments with casein and edestin in the living intestinal loop, could prove only the slightest absorption on excluding digestion as completely as possible, while the corresponding proteoses were abundantly absorbed.

It is difficult to decide in these experiments as to how far the proteins were taken up in an actually unchanged or partly modified form. The alimentary albuminuria observed repeatedly after the introduction of large quantities of protein into the intestinal canal speaks for an absorption of undigested protein under certain circumstances. To decide this question the biological method, using the precipitine reaction, has been made use of, and ASCOLI and VIGNO,[2] using this method, claim to have shown the passage of non-modified protein into the blood and lymph. Based upon many investigations on this subject we can consider it possible that under certain circumstances, as on flooding the intestinal canal with protein, with a greater permeability of the intestinal wall, as in new-born and sucking animals, and with a diminished modification by the gastric juice, a passage of non-modified protein may take place in the blood-vessels, but that under normal conditions this is not the case, or at least does not take place to any mentionable degree. As a rule, the absorption of protein follows a modification of the same, and the next question is whether the proteins are chiefly absorbed as proteoses or peptones or as simpler atomic complexes.

This question cannot be answered for the present. Investigations on the contents of the stomach and intestine have shown the presence of proteoses and peptones as well as non-biuret-giving atomic complexes and amino-acids. The results of the investigations of SCHMIDT-MÜLHEIM, ELLENBERGER and HOFMEISTER, EWALD and GUMLICH, ZUNZ, REACH, KUTSCHER and SEEMANN, ABDERHALDEN, GLAESSNER, and others[3] have given, as was to be expected, contradictory and variable results, and as the absorption runs more or less parallel with digestion the quantities of the various products found in the intestinal canal cannot give any positive conclusions as to the amounts produced.

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[1] Brücke, Wien. Sitzungsber., **59**; Bauer and Voit, Zeitschr. f. Biologie, **5**; Eichhorst, Pflüger's Arch., **4**; Czerny and Latschenberger, Virchow's Arch., **59**; Voit and Friedländer, Zeitschr. f. Biologie, **33**.

[2] Zeitschr. f. physiol. Chem., **39**.

[3] Schmidt-Mülheim, Arch. f. (Anat. u.) Physiol., 1879; Ellenberger and Hofmeister, *ibid.*, 1890; Ewald and Gumlich, Berlin. klin. Wochenschr., 1890; E. Zunz, Hofmeister's Beiträge, **3**; Reach, *ibid.*, **4**; Zunz, Annal. de la soc. roy. d. scienc. de Bruxelles, **13**; Kutscher and Seemann, Zeitschr. f. physiol. Chem., **34** and **35**; Abderhalden, *ibid.*, **44**; Glaessner, Zeitschr. f. klin. Med., **52**.

The proteoses, as well as the peptones, have been repeatedly found in the stomach and the intestine, and therefore the question has been raised for a long time how these bodies are absorbed and how they are introduced in the tissues. The generally accepted view is that they do not pass into the blood through the lymphatics, but through the intestinal epithelium, and this view is based essentially on the two following conditions: On completely isolating the chyle from the blood circulation, the protein absorption from the intestine is not impaired (LUDWIG and SCHMIDT-MÜLHEIM); and on a diet rich in protein the quantity thereof in the chyle (in man) was not noticeably increased (MUNK and ROSENSTEIN). ASHER and BARBÉRA[1] have shown in experiments on a dog that the quantity of protein in the lymph was slightly increased after partaking of considerable protein. This experiment does not disprove the assertion of MUNK that the blood-vessels form nearly the exclusive exit of the proteins from the intestinal tract.

After a diet rich in proteins neither proteoses nor peptones are found in the blood or the chyle. Nor are they present in the urine; and the absence of these bodies in the blood after digestion cannot be explained by the statement that they, like the proteoses (peptones) injected subcutaneously or directly into the blood, are quickly eliminated through the kidneys (PLÓSZ and GYERGYAI, HOFMEISTER, SCHMIDT-MÜLHEIM[2]). It might be supposed that the proteoses (peptones) formed in digestion are retained by the liver, and that this is the reason why they are not found in the blood. This explanation does not seem to be sufficient. NEUMEISTER has investigated the portal blood of rabbits into whose stomachs large quantities of proteoses and peptones had been introduced, without finding traces of the bodies in question.

He has also shown that when the liver of a dog is supplied with portal blood to which peptone is added (ampho-peptone), this is not retained by the liver. SHORE has arrived at similar results in regard to the importance of the liver, and has also shown that the spleen cannot transform peptone. Peptone seems to pass neither into the blood nor the chylous vessels, and the following observation of LUDWIG and SAL-

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[1] Schmidt-Mülheim, Arch. f. (Anat. u.) Physiol., 1877; Munk and Rosenstein, Virchow's Arch., **123**; Asher and Barbéra, Centralbl. f. Physiol., **11**, 403; Munk, *ibid.*, **11**, 585. See also Mendel, Amer. Journ. Physiol., **2**.

[2] Plósz and Gyergyai, Pflüger's Arch., **10**; Hofmeister, Zeitschr. f. physiol. Chem., **5**; Schmidt-Mülheim, Arch. f. (Anat. u.) Physiol., 1880.

VIOLI bears out this assumption. These investigators introduced a peptone solution into a double-ligatured, isolated piece of the small intestine, which was kept alive by passing defibrinated blood through it, and observed that the peptone disappeared from the intestine, but that the blood passing through did not contain any peptone. CATHCART and LEATHES[1] with their own experiments as a basis, give another interpretation of SALVIOLI's observations, namely, by the statement that the disappearance of the peptone from the loop of the intestine depends upon a hydrolysis of the same. On the other hand, they also found that no peptone was taken up by the circulating blood.

It must be remarked in connection with this view that, according to EMBDEN and KNOOP, and LANGSTEIN, proteoses sometimes occur in blood-serum, and also that NOLF[2] has found, after abundant absorption of proteoses from the intestine, a small amount in the blood. This occurrence of proteoses in the blood is not contradictory to the view that the chief quantity of proteoses and peptones does not pass from the intestine into the blood as such.

Many observations indicate that the proteoses and peptones are transformed in some way in the intestine or intestinal wall, and a retransformation of proteoses into protein is considered most plausible.

Certain investigators, such as v. OTT, NADINE POPOFF, and JULIA BRINCK,[3] are of the opinion that the proteoses and peptones are transformed into seralbumin before they pass into the walls of the digestive tract. This transformation is brought about by means of the epithelium-cells, as also by the vital activity of a fungus called by JULIA BRINCK *Micrococcus restituens*. No positive proofs have been presented to support this view.

The view that the transformation of the proteoses and peptones takes place after they have been taken up by the mucous membrane has better foundation. According to the observations of HOFMEISTER,[4] the walls of the stomach and the intestine are the only parts of the body in which proteoses (peptones) occur constantly during digestion, and the fact that proteoses (peptones) at the temperature of the body disappeared after a time from the excised but apparently still living mucous coat of the stomach, also confirm this.

This disappearance of proteoses is considered by HOFMEISTER as a transformation into ordinary protein. For such a transformation of proteoses in the mucosa of the stomach, Glaessner[1] has suggested new experimental evidence, while the Hofmeister school (Embden and Knoop) consider the regeneration of peptone into coagulable protein in the intestine as not proved.

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[1] Neumeister, Sitzungsber. d. phys.-med. Gesellsch. zu Würzburg, 1889, and Zeitschr. f. Biologie, 24; Shore, Journ. of Physiol., 11; Salvioli, Arch. f. (Anat. u. (Physiol., 1880, Suppl.; Cathcart and Leathes, Journ. of Physiol., 33.

[2] See Chapter VI, foot-note 1, p. 183.

[3] v. Ott, Arch. f. (Anat. u.) Physiol., 1883; Popoff, Zeitschr. f. Biologie, 25; Brinck, *ibid.*, 453.

[4] Zeitschr. f. physiol. Chem., 6, and Arch. f. exp. Path. u. Pharm., 19, 20, and 22.

According to Hofmeister the leucocytes, which are increased during digestion, play an important part in the transformation of the proteoses and peptones. They may in the first place take up the proteoses (peptones) and be the means of transporting them to the blood, and secondly by their growth, regeneration, and increase may stand in close relationship to the transformation and assimilation of the bodies. Heidenhain, who considers that the transformation of peptones into protein in the mucous membrane is positively settled, does not attribute so great an importance to the leucocytes in the absorption of the peptones, chiefly on the ground of comparative estimation of the quantity of absorbed peptones and leucocytes. He considers it as more probable that the reconversion of the peptones into protein takes place in the epithelium layers. This view is further corroborated by the investigations of Shore.[2]

On account of the discovery of erepsin by Cohnheim, the theory as to the absorption of proteins has taken another direction. There seems to be a tendency to lean towards the view that the proteoses and peptones are split in the intestine, or in the intestinal mucosa, into simpler bodies which do not give the biuret test and from which the proteins are regenerated. The question whether the active agent is erepsin or trypsin is only of secondary importance, as both of these enzymes split the proteoses and peptones alike.

According to the investigations of the Hofmeister school on pepsin digestion, and of Fischer and Abderhalden on trypsin digestion (see Chapter II), the disappearance of the biuret test does not indicate a complete cleavage of the proteins into amino-acids, since peptoids or polypeptides occur; consequently it is for the present not possible to say to what extent the proteins are broken down in the intestinal canal, and how far the amino-acids and more complex atomic groups not giving the biuret reaction are produced. It is just as difficult to state with positiveness, although feeding experiments with this in view have been carried out, how far a regeneration of protein from such abiuret peptides or from amino-acids is possible.

The possibility of keeping an animal for a certain time in nitrogenous equilibrium with abiuret digestion products was first demonstrated by Loewi. He fed dogs with an abiuret digestion mixture of pancreas tissue and kept them in nitrogenous equilibrium for more than a month. Henderson and Dean were also able in a bitch to observe nitrogenous equilibrium for at least a few days by feeding the abiuret products of the acid cleavage of meat, while Lesser, on the contrary, could not bring the animal in nitrogenous equilibrium by using fibrin digested with trypsin. These negative results not only confront the positive results of Loewi but also the observations of Abderhalden and Rona, as well as of Henriques and Hansen,[1] and there is no doubt that mice, rats, and dogs can be kept for at least a certain time in nitrogenous equilibrium with abiuret digestion products consisting in great part of monamino-acids. Of special interest is, no doubt, the fact that in the experiments of Abderhalden and Rona, as well as of Henriques and Hansen, the abiuret products obtained from casein by pancreatic digestion could protect the animal from loss of nitrogen, while the products obtained by acid hydrolysis of casein or a mixture of amino-acids corresponding to casein (Abderhalden and Rona) could not do this. Remarkable is the observation of Henriques and Hansen that the products (monamino-acids?) not precipitable by phosphotungstic acid could also cover the nitrogen loss. It is hardly possible to draw any positive conclusions from the above experiments as to the ability of the animal body to regenerate proteins by synthesis from abiuret digestion products. It is just as difficult to say whether and to what extent a synthesis of protein from the simple cleavage products takes place in the intestinal wall. Cathcart and Leathes found that when peptone or end-products of pancreatic digestion were absorbed by the intestinal loop the amount of nitrogenous substances in the blood not precipitated by tannic acid regularly increased, which seems to indicate that these simple cleavage products were taken up by the blood.

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[1] Hofmeister's Beiträge, **1**.

[2] Heidenhain, Pflüger's Arch., **43**; Shore, l. c.

The extent of the protein absorption is dependent essentially upon the kind of food introduced, since as a rule the protein substances from an animal source are much more completely absorbed than from a vegetable source. As proof of this the following observations are given: In his experiments on the utilization of certain foods in the intestinal canal of man Rubner found that with an exclusively animal diet, on partaking of an average of 738–884 grams of fried meat or 948 grams of eggs per day, the nitrogen deficit with the excrement was only 2.5–2.8 per cent of the total nitrogen introduced. With a strictly milk diet the results were somewhat unfavorable, since after partaking of 4100 grams of milk the nitrogen deficit increased to 12 per cent. The conditions are quite different with vegetable food, as shown by the researches of Meyer, Rubner, Hultgren and Landergren, who made experiments with various kinds of rye bread and found that the loss of nitrogen through the fæces amounted to 22–48 per cent. Experiments with other vegetable foods, and also the investigations of SCHUSTER, CRAMER, MEINERT, MORI,[1] and others on the utilization of foods with mixed diets, have led to similar results. With the exception of rice, wheat bread, and certain very finely divided vegetable foods, it is found in general that the nitrogen deficit by the fæces increases with a larger quantity of vegetable material in the food.

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[1] Loewi, Arch. f. exp. Path. u. Pharm., 48. See also Henderson and Dean, Amer. Journ. of Physiol., 9; Lesser, Zeitschr. f. Biologie, 45; Abderhalden and Rona, Zeitschr. f. physiol. Chem., 42, 44, and 47; Henriques and Hansen, *ibid.*, 48.

The reason for this is manifold. The large quantity of cellulose frequently present in vegetable foods impedes the absorption of proteins. The greater irritation produced by the vegetable food itself or by the organic acids formed in the fermentation in the intestinal canal causes a more violent peristalsis, which drives the contents of the intestine faster than otherwise along the intestinal canal. Another and most important reason is the fact that a part of the vegetable protein substances seem to be indigestible.

In speaking of the functions of the stomach we stated that after the removal or excision of this organ, an abundant digestion and absorption of proteins may take place. It is therefore of interest to learn how the digestion and absorption of proteins go on after the extirpation of the second protein-digesting organ, the pancreas. In this connection there are the observations on animals after complete or partial extirpation of the gland by MINKOWSKI and ABELMANN, SANDMEYER, V. HARLEY, after destroying the gland by ROSENBERG, and also in man after closing the pancreatic duct by HARLEY and DEUCHER.[2] In all these different cases such discrepant figures have been obtained for the utilization of the proteins—between 80 per cent after the apparently complete exclusion of pancreatic juice in man (DEUCHER) and 18 per cent after extirpation of the gland in dogs (HARLEY)—that one can hardly draw any clear conception as to the extent and importance of the trypsin digestion in the intestine. This is not to be wondered at, because one would expect that in such cases the other digestive fluids undergo variation, and indeed to various degrees in the different cases. ZUNZ and MAYER[3] have also found that in dogs (meat digestion) the tying of the pancreatic passages is essentially compensated for by an increased secretion of pepsin and other proteolytic enzymes, and that in this case the demolition of the protein in the stomach goes further than in a normal animal.

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[1] Rubner, Zeitschr. f. Biologie, **15**; Meyer, *ibid.*, **7**; Hultgren and Landergren, Nord. med. Arch., **21**; Schuster, in Voit's "Untersuch. d. Kost," etc., 142; Cramer, Zeitschr. f. physiol. Chem., **6**; Meinert, "Über Massennährung," Berlin, 1885; Kellner and Mori, Zeitschr. f. Biologie, **25**.

[2] Abelmann, "Uber die Ausnützung der Nahrungsstoffe nach Pankreasexstirpation" (Inaug.-Dissert. Dorpat, 1890), cited from Maly's Jahresber., **20**; Sandmeyer, Zeitschr. f. Biologie, **31**; Rosenberg, Pflüger's Arch., **70**; Harley, Journ. of Pathol. and Bacteriol., 1895; Deucher, Correspond. Blatt f. Schweiz. Aerzte, **28**.

[3] Mem. de l'Acad. roy. de médic. de Belg., **18**.

The carbohydrates are, it seems, chiefly absorbed as monosaccharides. Dextrose, levulose, and galactose are probably absorbed as such. The two disaccharides, saccharose and maltose, ordinarily undergo an inversion in the intestinal tract and are converted into dextrose and levulose. Lactose is also, at least in certain animals, inverted in the intestine. In other mature animals, on the contrary, if the lactase formation is not excited by milk food, the sugar is not inverted or only to a slight extent (VOIT and LUSK, WEINLAND, PORTIER, RÖHMANN and NAGANO), and it probably is absorbed as such in these animals if it does not undergo fermentation, or, as RÖHMANN and NAGANO[1] assumed, if it is not transformed in the intestinal mucosa in some unknown way. An absorption of non-inverted carbohydrates is not improbable, and according to OTTO and v. MERING[2] the portal blood contains besides dextrose a dextrin-like carbohydrate after a carbohydrate diet. A part of the carbohydrates is destroyed by fermentation in the intestine, with the formation of lactic and acetic acids and other bodies.

The different varieties of sugars are absorbed with varying degrees of rapidity, but as a general thing absorption occurs very quickly. This absorption takes place more quickly in the upper part of the intestine than in the lower part (RÖHMANN, LANNOIS and LÉPINE, RÖHMANN and NAGANO[3]). It is generally admitted that the simpler sugars are more quickly absorbed than the disaccharides, while the statements as to the absorption of the disaccharides differ somewhat (HÉDON, ALBERTONI, WAYMOUTH REID, RÖHMANN and NAGANO). There seems to be no doubt that lactose is absorbed more slowly than the two other disaccharides. According to the extensive experiments of RÖHMANN and NAGANO, saccharose is absorbed more quickly than maltose. NAGANO[4] contends that the pentoses are absorbed more slowly than hexoses.

On the introduction of starch even in very considerable quantities into the intestinal tract no dextrose passes into the urine, a condition which probably depends in this case upon the absorption and assimilation and the slow saccharification taking place simultaneously. If, on the contrary, large quantities of sugar are introduced at one time, then an elimination of sugar by the urine takes place, and this elimination of sugar is called

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[1] Voit and Lusk, Zeitschr. f. Biologie, 28; Röhmann and Nagano, Pflüger's Arch., 95, which contains the references to the literature.

[2] Otto, see Maly's Jahresber., 17; v. Mering, Arch. f. (Anat. u.) Physiol., 1877.

[3] Lannois et Lépine, Arch. de Physiol. (3), 1; Röhmann, Pflüger's Arch., 41; see also foot-note 1.

[4] In regard to the literature on the absorption of sugars, see foot-note 1.

alimentary glycosuria. In these cases the assimilation of the sugar and the absorption do not occur at the same time, hence the liver and the remaining organs do not have the necessary time to fix and utilize the sugar. This glycosuria may also in part be due to the fact that the introduction of considerable quantities of sugar forces this substance to be absorbed not only in the ordinary way through the blood-vessels to the liver (see below), but also in part by passing into the blood circulation through the lymphatic vessels, thus evading the liver.

That quantity of sugar to which we must raise the ingested substance in order to produce an alimentary glycosuria gives, according to HOFMEISTER,[1] the *assimilation limit* for that same sugar. This limit is different for various kinds of sugar; and it also varies for the same sugar not only in different animals, but also in different members of the same species, as also in the same individual under different circumstances. In general it can be said that in regard to the ordinary varieties of sugar, such as dextrose, levulose, saccharose, maltose, and lactose, the assimilation limit is highest for dextrose and lowest for lactose. It must be admitted that with an overabundant quantity of sugars in the intestinal tract the disaccharides do not have sufficient time for their complete inversion, and this has been directly shown by RÖHMANN and NAGANO. It is, therefore, not remarkable that also disaccharides have been found in the urine in cases of alimentary glycosuria.[2]

The investigations of LUDWIG and v. MERING and others have explained how the sugars enter into the blood-stream, namely, that they as well as other bodies soluble in water do not ordinarily pass over into the chylous vessels in measurable quantities, but are chiefly taken up by the blood in the capillaries of the villi and in this way pass into the mass of the blood. These investigations have been confirmed by observations of I. MUNK and ROSENSTEIN[3] on human beings.

The reason why the sugars and other soluble bodies do not pass over into the chylous vessels in appreciable quantity is, according to HEIDENHAIN,[4] to be found in the anatomical conditions, in the arrangement of the capillaries close under the layer of epithelium. Ordinarily these capillaries find the necessary time for the removal of the water and the solids dissolved in it. But when a large quantity of liquid, such as a sugar solution, is introduced into the intestine at once, this is not possible, and in these cases a part of the dissolved bodies passes into the chylous vessels and the thoracic duct (GINSBERG and RÖHMANN [1]).

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[1] Arch. f. exp. Path. u. Pharm., 25 and 26.

[2] For the literature in regard to the passage of various kinds of sugars into the urine, see C. Voit, Ueber die Glykogenbildung, Zeitschr. f. Biologie, 28, and F. Voit, foot-note 3, p. 293. See also Blumenthal, Zur Lehre von der Assimilationsgrenze der Zuckerarten, Inaug.-Dissert. 1903, Strassburg.

[3] v. Mering, Arch. f. (Anat. u.) Physiol., 1877; Munk and Rosenstein, Virchow's Arch., 123.

[4] Pflüger's Arch., 43, Suppl.

The introduction of larger quantities of sugar into the intestine at one time can readily cause a disturbance with diarrhœal evacuations of the intestine. If the carbohydrate is introduced in the form of starch, then very large quantities may be absorbed without causing any disturbance, and the absorption may be very complete. RUBNER found the following: On partaking 508–670 grams of carbohydrates, as wheat bread, per day the part not absorbed amounted to only 0.8–2.6 per cent. For peas, where 357–588 grams were eaten, the loss was 3.6–7 per cent, and for potatoes (718 grams) 7.6 per cent. CONSTANTINIDI found on partaking 367–380 grams of carbohydrates, chiefly as potatoes, a loss of only 0.4–0.7 per cent. In the experiments of RUBNER, as also of HULTGREN and LANDERGREN,[2] with rye bread the utilization of carbohydrates was less complete, and the loss in a few cases rose even to 10.4–10.9 per cent. It at least follows from the experiments made thus far that man can absorb more than 500 grams of carbohydrates per diem without difficulty.

We generally consider the pancreas as the most important organ in the digestion and absorption of amylaceous bodies, and it is a question how these bodies are absorbed after the extirpation of the pancreas. As on the absorption of proteins, so also on the absorption of starch, the observations have given variable results. In certain cases the absorption was not impaired, while in others it was, on the contrary, rather diminished, and with dogs devoid of pancreas it has been found that the absorption was decreased to 50 per cent of the starch partaken (ROSENBERG, CAVAZZANI [3]).

Emulsification used to be considered as of the greatest importance in the absorption of fats, and this emulsion occurs in the chyle on the introduction into the intestine of not only neutral fats, but also of fatty acids. The fatty acids do not exist as such in the emulsified fat of the chyle. The investigations of I. MUNK, later confirmed by others, have shown that the fatty acids undergo in great part a synthesis into neutral fats in the walls of the intestine, and are carried as such by the stream of chyle into the blood This synthesis seems to take place in the mucous membrane (MOORE). The experimental evidence thus far obtained for this assumption is not very conclusive.[4]

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[1] Ginsberg, Pflüger's Arch., 44; Röhmann, *ibid.*, 41.

[2] Rubner, Zeitschr. f. Biologie, 15 and 19; Constantinidi, *ibid.*, 23; Hultgren and Landergren, l. c.

[3] Cavazzani, Centralbl. f. Physiol., 7. See foot-note 1, p. 419; also Lombroso, Hofmeister's Beiträge, 8.

[4] Munk, Virchow's Arch., 80. See also v. Walther, Arch., f. (Anat. u.) Physiol., 1890; Minkowski, Arch. f. exp. Path. u. Pharm., 21; Frank, Zeitschr. f. Biologie, 36; Moore, see Biochem. Centralbl., 1, 741; Frank and Ritter, Zeitschr. f. Biologie, 47.

The assumption that the fat is absorbed chiefly as an emulsion is partly based on the abundance of emulsified fat in the chyle after feeding with fat, and partly on the fact that a fat emulsion is often found in the intestine after such food. As an abundant cleavage of neutral fats occurs in the intestinal canal, and also as the fatty acids do not occur in the chyle as such, but as emulsified fat after a synthesis with glycerine into neutral fats, it is to be doubted whether the emulsified fat of the chyle originates from an absorption of emulsified fat in the intestine or from a subsequent emulsification of neutral fats formed synthetically. This doubt has greater warrant in that FRANK[1] has shown that the fatty-acid ethyl ester is abundantly taken up by the chyle from the intestine, not as such, but as split-off fatty acids from which then the neutral emulsified fats of the chyle are formed.

The assumption of an absorption of fats as an emulsion contradicts the fact that an emulsion produced by means of soaps is not permanent in an acid liquid; hence we cannot consider as possible the presence of an emulsion in the intestine so long as it is acid. This difficulty is not too serious, as the reaction is often due to only carbonic acid and bicarbonates and also as found by KÜHNE and recently shown by MOORE and KRUMBHOLZ,[2] the proteins have a preserving action upon fat emulsions. The older views as to fat absorption were that the fat was absorbed as soaps, soluble in water, as well as finely emulsified fat, and this last form was considered as of the greatest importance. This view has recently undergone essential modifications, due to the work of MOORE and ROCKWOOD, and especially to the extensive work of PFLÜGER.[3]

MOORE and ROCKWOOD have shown the great solvent action of the bile for fatty acids, and on continuing these investigations further, MOORE and PARKER have found that the bile increases the solubility of soaps in water and can prevent their gelatinization, a fact which is of greater importance for the absorption of fats than the solubility of the fatty acids in bile. The quantity of lecithin in the bile is of great importance for the solubility therein of the fatty acids as well as the soaps. According to the above-mentioned investigators, the absorption of fat from the intestine is essentially dependent upon the solubility of the soaps and free fatty acids in the bile. The neutral fats are split and the free fatty acids are in part absorbed dissolved as such by the bile, and in part combined with alkalies, forming soaps. Neutral fats are regenerated from the fatty acids, and the alkali set free from the soaps is secreted back again into the intestine and used for the re-formation of soaps.

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[1] Zeitschr. f. Biologie, 36.

[2] Kühne, Lehrb. der physiol. Chem., 122; Moore and Krumbholz, Journ. of Physiol., 22.

[3] In regard to the newer literature on fat absorption, see the works of Pflüger, Pflüger's Arch., 80, 81, 82, 85, 88, 89, and 90, where the work of other investigators is cited and discussed.

The importance of the bile, the soaps, and the alkali carbonates has been closely studied, chiefly in the very thorough investigations of PFLÜGER. He has quantitatively determined the solvent power of the above-mentioned bodies—each alone as well as different mixtures of these—for the various fatty acids, and has closely studied the mode of action of the bile. From his investigations he has arrived at the conclusion that no unsplit fat is absorbed, that all fats, before their absorption, must first be split into glycerine and fatty acids, and that the bile, on account of its solvent power for soaps and fatty acids, is sufficient for the absorption of large quantities of fat eaten. The object of the formation of an emulsion is, according to this view, that the fat in this condition forms such a large surface for the action of the steapsin or the fat-splitting agents.

The possibility that all the fat must be first split and that no unsplit fat is absorbed is, according to these researches, not to be denied. It is the opinion of the author that it is still too early to give a positive verdict as to how these conditions in the intestine are brought about and the conclusion must be left for further investigations.

The next question is whether all the fat or the greater part of the same passes into the blood through the lymphatics and the thoracic duct. According to the researches of WALTHER and FRANK[1] on dogs, it seems that only a small part of the fats, or at least of the fatty acids fed passes into the chylous vessels; but these observations can hardly be applied to the absorption of neutral fats, or to the absorption in man under normal circumstances. MUNK and ROSENSTEIN,[2] in their investigations on a girl with a lymph fistula found 60 per cent of the fat ingested in the chyle, and of the total quantity of fat in the chyle only 4–5 per cent existed as soaps. On feeding with a foreign fatty acid,such as erucic acid, they found 37 per cent of the introduced body as neutral fat in the chyle.

The completeness with which fats are absorbed depends, under normal conditions, essentially upon the kind of fat. In this regard it is known, especially from the investigations of MUNK and ARNSCHINK,[3] that the varieties of fat with high melting-points, such as mutton-tallow and especially stearin, are not so completely absorbed as the fats with low melting points, such as hog- and goose-fat, olive-oil, etc. The kind of fat also has an influence upon the rapidity of absorption, as MUNK and ROSENSTEIN found that solid mutton-fat was absorbed more slowly than fluid lipanin. The extent of absorption in the intestinal tract is, under physiological conditions, very considerable. In the case of a dog investigated by VOIT it was found that out of 350 grams of fat (butter) partaken, 346 grams were absorbed from the intestinal canal, and according to the investigations of RUBNER[1] the human intestine can absorb over 300 grams of fat per diem. The fats are, according to RUBNER, much more completely absorbed when free, in the form of butter or lard, than when enclosed in cell-membranes, as in bacon.

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[1] Walther, Arch. f. (Anat. u.) Physiol., 1900; Frank, *ibid.*, 1892.

[2] Virchow's Arch., **123**.

[3] Munk, Virchow's Arch., **80** and **95**; Arnschink, Zeitschr. f. Biologie, **26**.

CLAUDE BERNARD showed long ago with experiments on rabbits in which the ductus choledochus was made to open into the small intestine above the pancreatic duct, that after food rich in fats the chylous vessels of the intestine above the pancreas passages were transparent, while below they were milk-white, and also that the bile alone cannot produce an absorption of the emulsified fat without the pancreatic juice. DASTRE[2] has performed the reverse experiment on dogs. He tied the ductus choledochus and adjusted a biliary fistula so that the bile flowed into the intestine below the mouth of the pancreatic passages. On killing the animal after a meal rich in fat the chylous vessels were first found milk-white below the discharge of the biliary fistula. From this DASTRE draws the conclusion that a combined action of the bile and pancreatic juice is important in the absorption of fats—a conclusion which stands in good accord with the experience of many others.

Through numerous observations of many investigators, such as BIDDER and SCHMIDT, VOIT, RÖHMANN, FR. MÜLLER, I. MUNK,[3] and others, it has been shown that the exclusion of the bile from the intestinal tract diminishes the absorption of fat to such an extent that only one seventh to about one half of the quantity of fat ordinarily absorbed undergoes absorption. In icterus with entire exclusion of the bile, a considerable decrease in the absorption of fat is noticed. As under normal conditions, so also in the absence of bile in the intestine, the lower-melting parts of the fat are more completely absorbed than those which have a high melting-point. I. MUNK found in his experiments on dogs with lard and mutton-tallow that the absorption of the high-melting tallow was reduced twice as much as the lard on the exclusion of the bile from the intestine.

We also learn from the investigations of RÖHMANN and I. MUNK that in the absence of bile the relationship between fatty acids and neutral fats is changed, namely, about 80–90 per cent of the fat existing in the fæces consists of fatty acid, while under normal conditions the fæces contain 1 part neutral fat to about 2–2½ parts free fatty acids. It is not possible to state how this increased quantity of fatty acids in the fat of the fæces is produced upon the exclusion of the bile from the intestine.

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[1] Voit, Zeitschr. f. Biologie, **9**; Rubner, *ibid.*, **15**.

[2] Arch. de Physiol. (5), **2**.

[3] F. Müller, Sitzungsber. der phys.-med. Gesellsch. zu Würzburg, 1885; I. Munk, Virchow's Arch., **122**. See also foot-notes 2 and 3, p. 406.

There is no doubt that the bile is of great importance in the absorption of fats. Still there is also no doubt that rather considerable quantities of fat may be absorbed from the intestine in the absence of bile. What relation does the pancreatic juice bear to this fact?

Upon this point a rather large number of observations on animals have been made by ABELMANN and MINKOWSKI, SANDMEYER, HARLEY, ROSENBERG, HÉDON and VILLE, and also on man by FR. MÜLLER and DEUCHER.[1] In all of these investigations a more or less diminished absorption of fat was observed after the extirpation or destruction of the gland, or the exclusion of the juice from the intestine. The results are very diverse as to the extent of this diminution, as in certain cases no absorption of fat was observed, while, in other cases, a considerable absorption was noted in the same class of animal (dog) and even in the same animal. According to MINKOWSKI and ABELMANN, after the total extirpation of the pancreas the fat of the food introduced is not absorbed at all, with the exception of milk, of which 28–53 per cent of the fat is absorbed. Other investigators have obtained other results, and HARLEY has observed a case where in a dog an absorption of only 4 per cent of the milk-fat, or, on the complete exclusion of intestinal bacteria, even no absorption, took place. The conditions may be somewhat different in the different cases; but it is certain that the absence of pancreatic juice from the intestine essentially affects the fat absorption. It is also just as certain that the absorption of fat is most abundant in the simultaneous presence of bile as well as pancreatic juice in the intestine. A little fat may still be absorbed even in the absence of these two fluids, as shown by the investigations of HÉDON and VILLE and CUNNINGHAM.[2]

The reason for the fact that the fat absorption is diminished in the absence of bile from the intestine must be sought for in the above-mentioned rôle of this fluid. It is more difficult to state why the absence of pancreatic juice causes a reduction in the absorption of fat. The most natural view is that the neutral fats are here less completely split, but this does not seem to be the case, because the non-absorbed fat of the fæces consists, on the exclusion of bile and pancreatic juice (MINKOWSKI and ABELMANN, HARLEY, HÉDON and VILLE, DEUCHER), chiefly of free fatty acids. A still unknown change caused by gastric lipase or by micro-organisms or otherwise may produce a cleavage of the fat in these cases. The imperfect fat absorption after the extirpation of the pancreas can possibly be explained by the removal of a considerable part of the alkalies necessary for the formation of the emulsion and for the solution of the fatty acids, but as SANDMEYER found in dogs deprived of their pancreas that the fat absorption was raised by giving chopped pancreas with the fat, this can hardly be a sufficient explanation.

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[1] Müller, "Unters. über den Icterus," Zeitschr. f. klin. Med., 12; Hédon and Ville, Arch. de Physiol. (5), 9; Harley, Journ. of Physiol., 18, Journ. of Pathol. and Bacteriol., 1895, and Proceed. Roy. Soc., 61. In regard to the other authors see foot-note 2, p. 418.

[2] Hédon and Ville, l. c.; Cunningham, Journ. of Physiol., 23.

The soluble salts are also absorbed with the water. The proteins, which can dissolve a considerable quantity of salts, such as earthy phosphates which are otherwise insoluble in alkaline water, are of great importance in the absorption of such salts.

The soluble constituents of the digestive secretions may, like other dissolved bodies, be absorbed, as is demonstrated by the passage of pepsin into urine; the enzymes may also be absorbed. The occurrence of urobilin in urine attests the absorption of the bile-constituents under physiological conditions despite the fact that the occurrence of very small traces of bile-acids in the urine is disputed. The absorption of bile-acids by the intestine seems to be positively proved by other observations. TAPPEINER[1] introduced a solution of bile-salts of a known concentration into an intestinal knot and after a time investigated the contents. He found that in the jejunum and the ileum, but not in the duodenum, an absorption of bile-acids took place, and further that of the two bile-acids only the glycocholic acid was absorbed in the jejunum. Further, SCHIFF long ago expressed the opinion that bile undergoes an intermediate circulation, in such wise that it is absorbed from the intestine, then carried to the liver by the blood, and lastly eliminated from the blood by this organ. Although this view has met with some opposition, still its correctness seems to be established by the researches of various investigators, and more recently by PREVOST and BINET, and specially by STADELMANN and his pupils.[2] After the introduction of foreign bile into the intestine of an animal the foreign bile-acids appear again in the secreted bile.

How does the removal of large portions of the various parts of the intestine affect absorption? HARLEY[3] has been able to perform a partial extirpation of the large intestine and in another instance a complete extirpation. This last condition increased the fæces considerably, especially because of the large increase in the water (fivefold). Fats and carbohydrates were absorbed just as completely as in the normal. The absorption of the proteins, on the contrary, was reduced to only 84 per cent as compared to 93–98 per cent in normal dogs. After extirpation the fæces sometimes did not contain any urobilin or only traces thereof, while bile-pigments existed in large amounts.

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[1] Wien. Sitzungsber., **77**.

[2] Schiff, Pflüger's Arch., **3**; Prevost and Binet, Compt rend., **106**; Stadelmann, see foot-note 1, p. 309.

[3] Proceed. Roy. Soc., **64**.

Erlanger and Hewlett[1] found that dogs, from which 70–83 per cent of the total length of the jejunum and ileum had been removed, could be kept alive like other animals if only the food was not too rich in fat. When the food contained large amounts of fat then 25 per cent was evacuated by the fæces as compared to 4–5 per cent in the normal animal. Under these same conditions the amount of nitrogen in the fæces was increased to twice the normal amount.

After the exclusion of the colon in rabbits, Bergmann and Hultgren[2] could find no definite action upon the availability of the cellulose and also no diminution in the utility of the other constituents of the food could be observed. Zuntz and Ustjanzew[3] also found that the removal of the cæcum had no influence on the utilization of nitrogen; but in regard to other points they arrived at different results. They found, namely, that the cæcum of the rodent is of great importance for the digestion of crude fibre and the pentosanes. On feeding hay and wheat to rabbits after the removal of the cæcum, the digestion coefficient for crude fibre fell from 42.8 to 23.4–18.7 per cent and for pentosanes from 50 to 40–28.7 per cent.

The question as to the forces which are active in the intestine during absorption has not been answered. It is certain that thus far the laws of diffusion and osmosis alone are not sufficient to explain absorption, although the views are disputed. With all these facts in view, and as it is not within the scope of this book to enter more in detail upon the numerous investigations on this subject, we must refer to larger works[4] and to text-books on physiology for further information.

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[1] Amer. Journ. of Physiol., 6.

[2] Skand. Arch. f. Physiol., 14.

[3] Verhandl. d. physiol. Gesellsch. zu Berlin, 1904–1905.

[4] See Höber, Physikalische Chemie der Zelle, Leipzig, 1906, and I. Munk, Ergebnisse der Physiologie, I, Abt. 1; Hamburger, Osmotischer Druck und Ionenlehre, Bd. 2, Wiesbaden, 1904.

CHAPTER X.

TISSUES OF THE CONNECTIVE SUBSTANCE.

I. The Connective Tissues.

THE form-elements of the typical connective tissues are cells of various kinds, of a not very well-known chemical composition, and gelatine-yielding fibrils, which, like the cells, are imbedded in an interstitial or intercellular substance. The fibrils consist of *collagen*. The interstitial substance contains chiefly *mucoid* (*tendon-mucoid*), besides *serglobulin* and *seralbumin*, which occur in the parenchymatous fluid (LOEBISCH [1]).

The connective tissue also often contains fibres or formations consisting of *elastin*, sometimes in such great quantities that the connective tissue is transformed into elastic tissue. A third variety of fibres, the reticular fibres, also occurs, and according to SIEGFRIED these consist of *reticulin*.

If finely divided tendons are extracted in cold water or NaCl solutions, the protein bodies soluble in the nutritive fluid in addition to a little mucoid are dissolved. If the residue is extracted with half-saturated lime-water, then the mucoid is dissolved and may be precipitated from the filtered extract by adding an excess of acetic acid. The extracted residue contains the fibrils of the connective tissue together with the cells and the elastic substance.

The so-called tendon mucin is not true mucin, but a mucoid, which, as first shown by LEVENE and then by CUTTER and GIES, contains a part of its sulphur as an acid related to chondroitin-sulphuric acid. These mucoids, which according to CUTTER and GIES are mixtures of several glucoproteids, contain 2.2–2.33 per cent sulphur, as shown by the analyses of CHITTENDEN and GIES, as well as those of CUTTER and GIES. The quantity of sulphur split off as sulphuric acid was 1.33–1.62 per cent (CUTTER and GIES [2]).

The fibrils of the connective tissue are elastic and swell slightly in water, somewhat more in dilute alkalies or in acetic acid. On the other hand, they shrink by the action of certain metallic salts, such as ferrous sulphate or mercuric chloride, and tannic acid, which form insoluble compounds with the collagen. Among these compounds, which prevent putrefaction of the collagen, that with tannic acid has been found of the greatest technical importance in the preparation of leather. In regard to the collagens, gelatines, elastins, and reticulins, see pages 75 to 81.

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[1] Zeitschr. f. physiol. Chem., **10**.

[2] Levene, *ibid.*, **31** and **39**; Cutter and Gies, Amer. Journ. of Physiol., **6**; Chittenden and Gies, Journ. of exp. Med., **1**.

The tissues described under the names *mucous* or *gelatinous tissues* are characterized more by their physical than by their chemical properties and have been but little studied. This much, however, is known, that the mucous or gelatinous tissues contain, at least in certain cases, as in the acalephæ, no mucin.

The umbilical cord is the most accessible material for the investigation of the chemical constituents of the gelatinous tissues. The mucin occurring therein has been described on page 67. C. Th. Mörner[1] has found a *mucoid* in the vitreous humor which contains 12.27 per cent nitrogen and 1.19 per cent sulphur.

Young connective tissue is richer in mucoid than old. Halliburton[2] found an average of 7.66 p. m. mucoid in the skin of very young children and only 3.85 p. m. in the skin of adults. In so-called myxœdema, in which a re-formation of the connective tissue of the skin takes place, the quantity of mucoid is also increased.

The connective tissue and also the elastic tissue are richer in water and poorer in solids in young animals as compared with full-grown animals. This may be seen from the following analyses of the Achilles tendon (Buerger and Gies) and of the ligamentum nuchæ (Vandegrift and Gies[3]).

	Achilles tendon.		Ligament.	
	Calf.	Ox.	Calf.	Ox.
Water	675.1 p. m.	628.7 p. m.	651.0 p. m.	575.7 p. m.
Solids	324.9 “	371.3 “	394.0 “	424.3 “
Organic bodies	318.4 “	366.6 “	342.4 “	419.6 “
Inorganic bodies	6.1 “	4.7 “	6.6 “	4.7 “
Fat	.....	10.4 “	..... “	11.2 “
Proteid	.....	2.2 “	.....	6.16 “
Mucoid	.....	12.83 “	.....	5.25 “
Elastin	.....	16.33 “	.....	316.70 “
Collagen	.....	315.88 “	.....	72.30 “
Extractives, etc	.....	8.96 “	.....	7.99 “

In regard to the mineral bodies it must be remarked that according to the determinations of H. Schulz[4] the connective tissue is rich in silicic acid. The greatest amount was found by him in the crystalline lens of the ox, namely, 0.5814 gram per kilo of dried substance. In man he found

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[1] Zeitschr. f. physiol. Chem., 18, 250.

[2] Mucin in Myxœdema: Further Analyses. King's College Collected Papers No. 1, 1893.

[3] Buerger and Gies, Amer. Journ. of Physiol., 6; Vandegrift and Gies, *ibid.*, 5.

[4] Pflüger's Arch., 84 and 89.

0.0637 gram in the tendons, 0.1064 gram in the fascia, and 0.244 gram in Wharton's jelly for every kilo of dried substance. The quantity of silicic acid is higher in the young than in the old; in man it is highest in the embryonic connective tissue of the umbilical cord. In the last-named substance SCHULZ found also 0.403 gram Fe_2O_3, 0.693 gram MgO, 3.297 grams CaO, and 3.794 grams P_2O_5 for every kilo of dried substance.

II. Cartilage.

Cartilaginous tissue consists of cells and an original hyaline matrix, which, however, may become changed in such wise that there appears in it a network of elastic fibres or connective-tissue fibrils.

Those cells that offer great resistance to the action of alkalies and acids have not been carefully studied. According to former views, the matrix was considered as consisting of a body analogous to collagen, so-called *chondrigen*. The recent investigations of MOROCHOWETZ and others, but especially those of C. MÖRNER,[1] have shown that the matrix of the cartilage consists of a mixture of collagen with other bodies.

The tracheal, thyroideal, cricoidal, and arytenoidal cartilages of full-grown cattle contain, according to MÖRNER, four constituents in the matrix, namely, *chondromucoid, chondroitin-sulphuric acid, collagen*, and an *albuminoid.*

Chondromucoid. This body, according to MÖRNER, has the composition C 47.30, H 6.42, N 12.58, S 2.42, O 31.28 per cent. Sulphur is in part loosely combined and may be split off by the action of alkalies, and a part separates as sulphuric acid when boiled with hydrochloric acid. Chondromucoid is decomposed by dilute alkalies and yields alkali albuminate, peptone substances, chondroitin-sulphuric acid, alkali sulphides, and some alkali sulphates. On boiling with acids it yields acid albuminate, peptone substances, chondroitin-sulphuric acid, and on account of the further decomposition of this last body, sulphuric acid and a reducing substance are formed.

Chondromucoid is a white, amorphous, acid-reacting powder which is insoluble in water, but dissolves easily on the addition of a little alkali. This solution is precipitated by acetic acid in great excess and by small quantities of mineral acids. The precipitation may be retarded by neutral salts or by chondroitin-sulphuric acid. The solution containing NaCl and acidified with HCl is not precipitated by potassium ferrocyanide. Precipitants for chondromucoid are alum, ferric chloride, sugar of lead, or basic lead acetate. Chondromucoid is not precipitated by tannic acid, and it may by its presence prevent the precipitation of gelatine by this acid. It gives the usual color reactions for proteins, namely, with nitric acid, with copper sulphate and alkali, with Millon's and Adamkiewicz's reagents.

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[1] Morochowetz, Verhandl. d. naturh. med. Vereins zu Heidelberg, **1**, Heft 5; Mörner, Skand. Arch. f. Physiol., **1**.

Chondroitin-sulphuric Acid, chondroitic acid. This acid, which was first prepared pure from cartilage by C. Mörner and identified by him as an ethereal sulphuric acid, occurs, according to Mörner, in all varieties of cartilage and also in the tunica intima of the aorta and as traces in the bone substance. K. Mörner has also found it in the ox-kidney and in human urine as a regular constituent. According to Krawkow, who found it in the cervical ligament of the ox, it combines with proteid, forming amyloid (see page 69), which explains the occurrence of this body in amyloid-degenerated livers, as observed by Oddi.[1] The identity of the ethereal sulphuric acid occurring in liver amyloid with chondroitin-sulphuric acid does not seem to be quite clear, according to the researches of Monéry. According to Levene,[2] the glucothionic acid which is prepared from tendon mucoid and which gives the orcin reaction for glucuronic acid, and yields furfurol on distillation with hydrochloric acid, is not identical with the chondroitin-sulphuric acid, but is probably related thereto.

Chondroitin-sulphuric acid has the formula $C_{18}H_{27}NSO_{17}$, according to Schmiedeberg.[3] As primary products this acid yields on cleavage sulphuric acid and a nitrogenous substance, *chondroitin*, according to the following equation:

$$C_{18}H_{27}NSO_{17}+H_2O=H_2SO_4+C_{18}H_{27}NO_{14}.$$

Chondroitin, which is similar to gum arabic and which is a monobasic acid, yields acetic acid and a new nitrogenous substance, *chondrosin*, as cleavage products, on decomposition with dilute mineral acids:

$$C_{18}H_{27}NO_{14}+3H_2O=3C_2H_4O_2+C_{12}H_{21}NO_{11}.$$

Chondrosin, which is also a gummy substance soluble in water, is a monobasic acid and reduces copper oxide in alkaline solution even more strongly than dextrose. It is dextrogyrate and represents the reducing substance obtained by previous investigators in an impure form on boiling cartilage with an acid. The products obtained on decomposing chondrosin with barium hydrate tend to show, according to Schmiedeberg, that chondrosin contains the atomic groups of glucuronic acid and glucosamine. This assumption does not seem to have sufficient foundation. According to Ogler and Neuberg,[4] chondrosin does not give the orcin test nor does it yield furfurol. It contains neither glucuronic acid nor glucosamine, and on cleavage with baryta it yields, besides a carbohydrate complex which has not been studied, an oxyamino-acid having the formula $C_6H_{13}O_6N$; also a hexosamine acid or tetraoxyaminocaproic acid.

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[1] C. Mörner, l. c., and Zeitschr. f. physiol. Chem., **20** and **23**; K. Mörner, Skand. Arch. f. Physiol., **6**; Krawkow, Arch. f. exp. Path. u. Pharm., **40**; Oddi, *ibid.*, **33**.

[2] Monéry, Compt. rend. soc. biol., **54**; Levene, Zeitschr. f. physiol. Chem., **39**.

[3] Arch. f. exp. Path. u. Pharm., **28**.

[4] Zeitschr. f. physiol. Chem., **37**.

Chondroitin-sulphuric acid appears as a white amorphous powder. which dissolves very easily in water, forming an acid solution and, when sufficiently concentrated, a sticky liquid similar to a solution of gum arabic. Nearly all of its salts are soluble in water. The neutralized solution is precipitated by tin chloride, basic lead acetate, neutral ferric chloride, and by alcohol in the presence of a little neutral salt. The solution, on the other hand, is not precipitated by acetic acid, tannic acid, potassium ferrocyanide and acid, sugar of lead, mercuric chloride, or silver nitrate. Acidified solutions of alkali chondroitin-sulphates cause a precipitation when added to solutions of gelatine or proteid.

Chondromucoid and chondroitin-sulphuric acid may be prepared, according to Mörner, by extracting finely cut cartilage with water, which dissolves the preformed chondroitin-sulphuric acid besides some chondromucoid. In this watery extract, the chondroitin-sulphuric acid prevents the precipitation of the chondromucoid by means of an acid. If 2–4 p. m. HCl is added to this watery extract and warmed on the water-bath, the chondromucoid gradually separates, while the chondroitin-sulphuric acid and the rest of the chondromucoid remain in the filtrate. If the cartilage, which has been lixiviated with water, at the temperature of the body, is extracted with hydrochloric acid of 2–3 p. m. until the collagen is converted into gelatine and dissolved, the remaining chondromucoid may be removed from the insoluble residue by dilute alkali and precipitated from the alkaline extract by an acid. It may be purified by repeated solution in water with the aid of a little alkali, and precipitation with an acid, and then finally by extraction with alcohol and ether.

The pre-existing chondroitin-sulphuric acid, or that formed by the decomposition of chondromucoid, is obtained by lixiviating the cartilage with a 5 per cent caustic-alkali solution. The alkali albuminate formed by the decomposition of the chondromucoid can be removed from the solution by neutralization, then the peptone precipitated by tannic acid, the excess of this acid removed with sugar of lead, and the lead separated from the filtrate by H_2S. If further purification is necessary, the acid is precipitated with alcohol, the precipitate dissolved in water, this solution dialyzed and precipitated again with alcohol,—this solution in water and precipitation with alcohol being repeated a few times,—and lastly the acid is treated with alcohol and ether.

Schmiedeberg prepared the acid from the septum narium of the pig according to the following method: The finely divided cartilage is first exposed to artificial peptic digestion, then carefully washed with water and the insoluble residue treated with 2–3 per cent hydrochloric acid. This cloudy liquid containing hydrochloric acid is precipitated with alcohol (about $\frac{1}{4}$ vol.) and the clear filtrate treated with absolute alcohol and some ether. The precipitate, consisting chiefly of a combination or a mixture of chondroitin-sulphuric acid and gelatine peptone (peptochondrin), is first washed with alcohol and then with water. It is then dissolved in alkaline water and the basic alkali compound precipitated from this solution by the addition of alcohol, whereby the gelatine-peptone alkali remains in solution. The precipitate is purified by repeated solution in alkaline water and precipitated by alcohol. To obtain chondroitin-sulphuric acid entirely free from chondroitin it is more advantageous to prepare the potassium-copper compound of the acid from the alkaline solution by the alternate addition of copper acetate and caustic potash and precipitation with alcohol. The reader is referred to the original article for more details and also for ODDI's method.

The *collagen* of the cartilage gives, according to MÖRNER, a gelatine which contains only 16.4 per cent N and which can hardly be considered identical with ordinary gelatine.

In the above-mentioned cartilages of full-grown animals the chondroitin-sulphuric acid and chondromucoid, perhaps also the collagen, are found surrounding the cells as round balls or lumps. These balls (MÖRNER'S *chondrin-balls*), which give a blue color with methyl-violet, lie in the meshes of a trabecular structure, which is colored when brought in contact with tropæolin.

The *albuminoid* is a nitrogenized body which contains loosely combined sulphur. It is soluble with difficulty in acids and alkalies and resembles keratin in many respects, but differs from it by being soluble in gastric juice. In other respects it is more similar to elastin, but differs from this substance by containing sulphur. This albuminoid gives the color reactions of the protein bodies.

The preparation of cartilage gelatine and the albuminoid may be performed according to the following method of MÖRNER: First remove the chondromucoid and chondroitin-sulphuric acid by extraction with dilute caustic potash (0.2–0.5 per cent), remove the alkali from the remaining cartilage by water, and then boil with water in a PAPIN'S digester. The collagen passes into solution as gelatine, while the albuminoid remains undissolved (contaminated by the cartilage-cells). The gelatine may be purified by precipitating with sodium sulphate, which must be added to saturation in the faintly acidified solution, redissolving the precipitate in water, dialyzing well, and precipitating with alcohol.

According to MÖRNER, no albuminoid is found in young cartilage, but only the three first-mentioned constituents. Nevertheless the young cartilage contains about the same amounts of nitrogen and mineral substances as the old. The cartilage of the ray (*Raja batis* LIN.), which has been investigated by LÖNNBERG,[1] contains no albuminoid and only a little chondromucoid, but a large proportion of chondroitin-sulphuric acid and collagen.

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[1] Maly's Jahresber., 19, 325.

According to PFLÜGER and HÄNDEL,[1] glycogen occurs to a slight extent in all matrices, and of these it is richest in the cartilage. Tendons, ligamentum nuchæ, and cartilage of the ox contained 0.06, 0.07, and 2.17 p. m. glycogen respectively (HÄNDEL).

HOPPE-SEYLER found in fresh human rib-cartilage 676.7 p. m. water, 301.3 p. m. organic and 22 p. m. inorganic substance, and in the cartilage of the knee-joint 735.9 p. m. water, 248.7 p. m. organic and 15.4 p. m. inorganic substance. PICKARDT found 402–574 p. m. water and 72.86 p. m. ash (no iron) in the laryngeal cartilage of oxen. The ash of cartilage contains considerable amounts (even 800 p. m.) of alkali sulphate, which probably does not exist originally as such, but is produced in great part by the incineration of the chondroitin-sulphuric acid and the chondromucoid. The analyses of the ash of cartilage therefore cannot give a correct idea of the quantity of mineral bodies existing in this substance. The cartilage is richest in sodium of all the tissues of the body, and according to BUNGE[2] the amount of Na and Cl is greatest in young animals. In 1000 parts of cartilage dried at 120° C., BUNGE found 91.26 parts Na_2O in the shark, 33.98 in the ox embryo, 32.45 in a fourteen-day-old calf, and 26.4 in a ten-weeks-old calf.

The Cornea. The corneal tissue, which is considered by many investigators to be related to cartilage in a chemical sense, contains traces of proteid and a *collagen* as chief constituent, which C. MÖRNER[3] claims contains 16.95 per cent N. According to him it also contains a *mucoid* which has the composition C 50.16, H 6.97, N 12.79, and S 2.07 per cent. On boiling with dilute mineral acid this mucoid yields a reducing substance. The globulins found by other investigators in the cornea are not derived from the matrix, according to MÖRNER, but from the layer of epithelium. According to MÖRNER, DESCEMET's membrane consists of *membranin* (page 69), which contains 14.77 per cent N and 0.90 per cent S.

In the cornea of oxen HIS[4] found 758.3 p. m. water, 203.8 p. m. gelatine-forming substance, 28.4 p. m. other organic substance, besides 8.1 p. m. soluble and 1.1 p. m. insoluble salts.

III. Bone.

The bony structure proper, when free from other formations occurring in bones, such as marrow, nerves, and blood-vessels, consists of cells and a matrix.

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[1] Pflüger's Arch., 92; Händel, *ibid.*

[2] Hoppe-Seyler, cited from Kühne's Lehrbuch d. physiol. Chem., 387; Pickardt, Centralbl. f. Physiol., 6, 735; Bunge, Zeitschr. f. physiol. Chem., 28.

[3] Zeitschr. f. physiol. Chem., 18.

[4] Cited from Gamgee, Physiol. Chem., 1880, 451.

The *cells* have not been closely studied in regard to their chemical constitution. On boiling with water they yield no gelatine. They contain no keratin, which is not usually present in the bony structure (Herbert Smith[1]).

The *matrix* of the bony structure contains two chief constituents, namely, an organic substance, and the so-called *bone-earths*, lime-salts, enclosed in or combined with it. If bones are treated with dilute hydrochloric acid at the ordinary temperature, the lime-salts are dissolved and the organic substance remains as an elastic mass, preserving the shape of the bone.

The organic matrix consists chiefly of *ossein*, which is generally considered as identical with the collagen of the connective tissue. It also contains, as Hawk and Gies[2] have shown, *mucoid* and *albuminoid*. After the removal of the lime-salts by hydrochloric acid of 2–5 p. m. these experimenters were able to extract the mucoid by one-half saturated lime-water and to precipitate it with 2 p. m. hydrochloric acid. After the removal of the osseomucoid and collagen (by boiling with water) they obtained the albuminoid as an insoluble residue.

The osseomucoid on boiling with hydrochloric acid yielded a reducing substance and sulphuric acid, 1.11 per cent sulphur appearing in this form. The osseomucoid stands close to the chondro- and tendon mucoid in elementary composition, as may be seen from the following analyses:

	C	H	N	S	O	
Osseomucoid......	47.43	6.63	12.22	2.32	31.40	(Hawk and Gies)
Chondromucoid...	47.30	6.42	12.58	2.42	31.28	(C. Mörner)
Tendon mucoid...	48.76	6.53	11.75	2.33	30.60	(Chittenden and Gies)
Corneal mucoid..	50.16	6.97	12.79	2.07	28.01	(C. Mörner)

The osseoalbuminoid is insoluble in 2 p. m. hydrochloric acid and in 5 p. m. Na_2CO_3, but dissolves in 10 per cent KOH with the formation of albuminates. The composition of chondro- and osseoalbuminoid is as follows:

	C	H	N	S	O	
Osseoalbuminoid.........	50.16	7.03	16.17	1.18	25.46	Hawk and Gies
Chondroalbuminoid.......	50.46	7.05	14.95	1.86	25.68	

The inorganic constituents of the bony structure, the so-called *bone-earths*, which after the complete calcination of the organic substance remain as a white, brittle mass, consist chiefly of calcium and phosphoric acid, but also contain carbonic acid and, in smaller amounts, magnesium, chlorine, and fluorine. Alkali sulphate and iron, which have been found in bone-ash, do not seem to belong exactly to the bony substance, but to the nutritive fluids or to the other constituents of bones. The traces of sulphate occurring in the bone-ash are derived, according to **Mörner**,[1] from the chondroitin-sulphuric acid. According to **Gabriel**, potassium and sodium are essential constituents of bone-earth, and this has been substantiated by **Aron**.[2]

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[1] Zeitschr. f. Biologie, 19.

[2] Amer. Journ. of Physiol., 5 and 7.

The opinions of investigators differ somewhat as to the manner in which the mineral bodies of the bony structure are combined with each other. Chlorine is present in the same form as in apatite ($CaCl_2,3Ca_3P_2O_8$). If we eliminate the magnesium, the chlorine, and the fluorine, the last, according to **Gabriel**, occurring only as traces, the remaining mineral bodies form the combination $3(Ca_3P_2O_8)CaCO_3$. According to **Gabriel** the simplest expression for the composition of the ash of bones and teeth is ($Ca_3(PO_4)_2 + Ca_5HP_3O_{13} + Aq$), in which 2–3 per cent of the lime is replaced by magnesia, potash, and soda, and 4–6 per cent of the phosphoric acid by carbonic acid, chlorine, and fluorine.

Analyses of bone-earths have shown that the mineral constituents exist in rather constant proportions, which are nearly the same in different animals. As an example of the composition of bone-earth we here give the analyses of **Zalesky**.[3] The figures represent parts per thousand.

	Man.	Ox.	Tortoise.	Guinea-pig.
Calcium phosphate, $Ca_3P_2O_8$	838.9	860.9	859.8	873.8
Magnesium phosphate, $Mg_3P_2O_8$	10.4	10.2	13.6	10.5
Calcium combined with CO_2, Fl, and Cl	76.5	73.6	63.2	70.3
CO_2	57.3	62.0	52.7	....
Chlorine	1.8	2.0	.....	1.3
Fluorine [4]	2.3	3.0	2.0	....

Some of the CO_2 is always lost on calcining, so that the bone-ash does not contain the entire CO_2 of the bony substance.

Ad. Carnot[5] found the following composition for the bone-ash of man, ox, and elephant:

	Man.		Ox.	Elephant.
	Femur (body).	Femur (head).	Femur.	Femur.
Calcium phosphate	874.5	878.7	857.2	900.3
Magnesium phosphate	15.7	17.5	15.3	19.6
Calcium fluoride	3.5	3.7	4.5	4.7
Calcium chloride	2.3	3.0	3.0	2.0
Calcium carbonate	101.8	92.3	119.6	72.7
Iron oxide	1.0	1.3	1.3	1.5

The quantity of organic substance in the bones, calculated from the loss of weight in burning, varies somewhat between 300 and 520 p. m. This variation may in part be explained by the difficulty in obtaining the bony substance entirely free from water and partly by the very variable amount of blood-vessels, nerves, marrow, and the like in different bones. The unequal amounts of organic substance found in the compact and in the spongy parts of the same bone, as well as in bones at different periods of development in the same animal, depend probably upon the varying quantities of these above-mentioned tissues. *Dentin*, which is comparatively pure bony structure, contains only 260–280 p. m. organic substance, and HOPPE-SEYLER[1] therefore thinks it probable that perfectly pure bony substance has a constant composition and contains only about 250 p. m. organic substance. The question whether these substances are chemically combined with the bone-earths or only intimately mixed has not been decided.

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[1] Zeitschr. f. physiol. Chem., 23.

[2] Gabriel, *ibid.*, 18, which also contains the pertinent literature; Aron, Pflüger's Arch., 106.

[3] Hoppe-Seyler, Med.-chem. Untersuch., p. 19.

[4] The statements as to the quantity of fluorine are contradictory; see Harms, Zeitschr. f. Biologie, 38; Jodblauer, *ibid.*, 41.

[5] Compt. rend., 114.

The nutritive fluids which circulate through the bones have not been isolated, and we only know that they contain some protein and some NaCl and alkali sulphate. The yellow marrow contains chiefly fat, which consists of olein, palmitin, and stearin, and which differs from the fat of the other parts of the body by having a higher acetyl equivalent (ZINK[2]). Protein has been found especially in the so-called red marrow of the spongy bones. According to FORREST, the protein consists of a globulin coagulating at 47–50° C. and a nucleoalbumin with 1.6 per cent phosphorus (HALLIBURTON[3]), besides traces of albumin. Besides this the marrow contains so-called extractive bodies, such as lactic acid, hypoxanthine, and cholesterin, but mostly bodies of an unknown character.

The diverse quantitative composition of the various bones of the skeleton depends probably on the varying quantities of other tissues, such as marrow, blood-vessels, etc., which they contain. The same reason explains, to all appearances, the larger quantity of organic substance in the spongy parts of the bones as compared with the more compact parts. SCHRODT[4] has made comparative analyses of different parts of the skeleton of the same animal (dog) and has found an essential difference. The quantity of water in the fresh bones varies between 138 and 443 p. m. The bones of the extremities and the skull contain 138–222, the vertebræ 168–443, and the ribs 324–356 p. m. water. The quantity of fat varies between 13 and 269 p. m. The largest amount of fat, 256–269 p. m., is found in the long tubular bones, while only 13–175 p. m. fat is found in the small short bones. The quantity of organic substance, calculated from fresh bones, was 150–300 p. m., and the quantity of mineral substances 290–563 p. m. Contrary to the general supposition the greatest amount of bone-earths was not found in the femur, but in the first three cervical vertebræ. In birds the tubular bones are richer in mineral substances than the flat bones (DÜRING), and the greatest quantity of mineral bodies has been found in the humerus (HILLER, DÜRING [1]).

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[1] Physiol. Chem., 102–104.

[2] See Chem. Centralbl., 1897, I, 296.

[3] Forrest, Journ. of Physiol., 17; Halliburton, *ibid.*, 18.

[4] Cited from Maly's Jahresber., 6.

We do not possess trustworthy statements in regard to the composition of bones at different ages. The analyses by E. VOIT of bones of dogs and by BRUBACHER of bones of children apparently indicate that the skeleton becomes poorer in water and richer in ash with increase in age. GRAFFENBERGER [2] has found in rabbits 6½–7½ years old that the bones contained only 140–170 p. m. water, while the bones of the full-grown rabbit 2–4 years old contained 200–240 p. m. The bones of old rabbits contain more carbon dioxide and less calcium phosphate.

The composition of bones of animals of different species is but little known. The bones of birds contain, as a rule, somewhat more water than those of mammalia, and the bones of fishes contain the largest quantity of water. The bones of fishes and amphibians contain a greater amount of organic substance. The bones of pachyderms and cetaceans contain a large proportion of calcium carbonate; those of granivorous birds always contain silicic acid. The bone-ash of amphibians and fishes contains sodium sulphate. The bones of fishes seem to contain more soluble salts than the bones of other animals.

A great many experiments have been made to determine the exchange of material in the bones—for instance, with food rich in lime and with food deficient in lime—but the results have always been doubtful or contradictory. The attempts, also, to substitute other alkaline earths or alumina for the lime of the bones have given contradictory results.[3] On feeding sufficient calcium and phosphorus in the food ARON [4] found, by strongly reducing the sodium and at the same time giving a large amount of potassium, that the development of the bones was below normal. On the administration of madder the bones of the animal are found to be colored red after a few days or weeks; but these experiments have not led to any positive conclusion in regard to the growth or metabolism in the bones.

Under pathological conditions, as in rachitis and softening of the bones, an ossein has been found which does not give any typical gelatine on boiling with water. Otherwise pathological conditions seem to affect chiefly the quantitative composition of the bones, and especially the relationship between the organic and the inorganic substance. In exostosis and osteosclerosis the quantity of organic substance is generally increased. In rachitis and osteomalacia the quantity of bone-earths is considerably decreased. Attempts have been made to produce rachitis in animals by the use of food deficient in lime. From experiments on fully developed animals contradictory results have been obtained. In young, undeveloped animals ERWIN VOIT[1] produced, by lack of lime-salts, a change similar to rachitis. In full-grown animals, the bones were changed after a long time because of the lack of lime-salts in the food, but did not become soft, only thinner (osteoporosis). The attempts to remove the lime-salts from the bones by the addition of lactic acid to the food have led to no positive results (HEITZMANN, HEISS, BAGINSKY[2]). WEISKE, on the contrary, has shown, by administering dilute sulphuric acid or monosodium phosphate with the food (presupposing that the food gave no alkaline ash) to sheep and rabbits, that the quantity of mineral bodies in the bones might be diminished. On feeding continuously for a long time with a food which yielded an acid ash (cereal grains), WEISKE has observed a diminution in the mineral substances of the bones in full-grown herbivora.[3] A few investigators are of the opinion that in rachitis, as in osteomalacia, a solution of the lime-salts by means of lactic acid takes place. This was suggested by the fact that O. WEBER and C. SCHMIDT[4] found lactic acid in the cyst-like, altered bony substance in osteomalacia.

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[1] Hiller, cited from Maly's Jahresber., **14**; Düring, Zeitschr. f. physiol. Chem., **23**.

[2] Voit, Zeitschr. f. Biologie, **16**; Brubacher, *ibid.*, **27**; Graffenberger in Maly's Jahresber., **21**.

[3] See H. Weiske, Zeitschr. f. Biologie, **31**.

[4] Pflüger's Arch., **106**.

Well-known investigators have disputed the possibility of the lime-salts being washed from the bones in osteomalacia by means of lactic acid. They have given special prominence to the fact that the lime-salts held in solution by the lactic acid must be deposited on neutralization of the acid by the alkaline blood. This objection is not very important, as the alkaline blood-serum has the property to a high degree of holding earthy phosphates in solution, which fact can be easily proved. The investigations of LEVY[5] contradict the statement as to the solution of the lime-salts by lactic acid in osteomalacia. He has found that the normal relationship $6PO_4:10Ca$ is retained in all parts of the bones in osteomalacia, which would not be the case if the bone-earths were dissolved by an acid. The decrease in phosphate occurs in the same quantitative relationship as the carbonate, and according to LEVY, in osteomalacia the exhaustion of the bone takes place by a decalcification in which one molecule of phosphate carbonate after the other is removed.

In rachitis the quantity of organic matter has been found to vary between 664 and 811 p. m. The quantity of inorganic substance was 189–336 p. m. These figures refer to the dried substance. According to BRUBACHER, rachitic bones are richer in water than the bones of healthy children, and poorer in mineral bodies, especially calcium phosphate. In opposition to rachitis, osteomalacia is often characterized by the considerable amount of fat in the bones, 230–290 p. m.; but as a rule the composition varies so much that the analyses are of little value. In a case of osteomalacia, CHABRIE [1] found a larger quantity of magnesium than calcium in a bone. The ash contained 417 p. m. phosphoric acid, 222 p. m. lime, 269 p m. magnesia, and 86 p. m. carbon dioxide. Other investigators have on the contrary found considerably more calcium than magnesium.

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[1] Zeitschr. f. Biologie, 16.

[2] Heitzmann, Maly's Jahresber., 3, 229; Heiss, Zeitschr. f. Biologie, 12; Baginsky, Virchow's Arch., 87.

[3] See Maly's Jahresber., 22; also Weiske, Zeitschr. f. physiol. Chem., 20, and Zeitschr. f. Biologie, 31.

[4] Cited from v. Gorup-Besanez, Lehrb. d. physiol. Chem., 4. Aufl.

[5] Zeitschr. f. physiol. Chem., 19.

The **tooth-structure** is nearly related, from a chemical standpoint, to the bony structure.

Of the three chief constituents of the teeth—dentin, enamel, and cement—the *cement* is to be considered as true bony structure, and as such has already been discussed to some extent. *Dentin* has the same composition as the bony structure, but contains somewhat less water. The organic substance yields gelatine on boiling; but the dental tubes are not dissolved, therefore they cannot consist of collagen. In dentin 260–280 p. m. organic substance has been found. *Enamel* is an epithelium formation containing a large proportion of lime-salts. Corresponding to its character and origin, the organic substance of the enamel does not yield any gelatine. Completely developed enamel contains the least water, the greatest quantity of mineral substances, and is the hardest of all the tissues of the body. In full-grown animals it contains hardly any water, and the quantity of organic substance amounts to only 20–40–68 p. m. The relative amounts of calcium and phosphoric acid are, according to the analyses of HOPPE-SEYLER, about the same as in bone-earths. The quantity of chlorine according to HOPPE-SEYLER is remarkably high, 0.3–0.5 per cent, while BERTZ [2] found that the ash of enamel was free from chlorine and that dentin was very poor in chlorine.

CARNOT,[3] who has investigated the dentin from elephants, has found 4.3 p. m. calcium fluoride in the ash. In ivory he found only 2 p. m. Dentin from elephants is rich in magnesium phosphate, which is still more abundant in ivory.

According to GABRIEL the amount of fluorine is very small and amounts to 1 p. m. in ox-teeth. It is no greater in the teeth and enamel than in the bones.[4] The same investigator found that the amount of phosphates is strikingly small in the enamel, and in the teeth considerable lime is replaced by magnesia. This coincides with BERTZ's findings, that dentin contains twice as much magnesia as the enamel.

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[1] Chabrié, Les phénomènes chim. de l'ossification, Paris, 1895, 65.

[2] See Maly's Jahresber., **30**.

[3] Compt. rend., **114**.

[4] See foot-note 4, p. 436.

IV. The Fatty Tissue.

The membranes of the fat-cells withstand the action of alcohol and ether. They are not dissolved by acetic acid nor by dilute mineral acids, but are dissolved by artificial gastric juice. They may possibly consist of a substance closely related to elastin. The fat-cells contain, besides fat, a yellow pigment which in emaciation does not disappear so rapidly as the fat; and this is the reason that the subcutaneous cellular tissue of an emaciated corpse has a dark orange-red color. The cells deficient in or nearly free from fat, which remain after the complete disappearance of the latter, seem to have an albuminous protoplasm rich in water. Adipose tissue is rich in a fat-splitting enzyme and in catalases (see Chapter I).

The less water the fatty tissue contains the richer it is in fat. SCHULZE and REINECKE[1] found in 1000 parts

	Water.	Membrane.	Fat.
Fatty tissue of oxen	99.7	16.6	883.7
" " " sheep	104.8	16.4	878.8
" " " pigs	64.4	13.6	922.0

The fat contained in the fat-cells consists chiefly of triglycerides of stearic, palmitic, and oleic acids. Besides these, especially in the less solid kinds of fats, there are glycerides of other fatty acids (see Chapter IV). In all animal fats there are besides these, as FR. HOFMANN[2] has shown, also free, non-volatile fatty acids, although in very small amounts.

Human fat is relatively rich in olein, the quantity in the subcutaneous fatty tissue being 70–80 per cent or more.[3] In new-born infants it is poorer in oleic acid than in adults (KNÖPFELMACHER, SIEGERT, JAECKLE); the quantity of olein increases until the end of the first year, when it is about the same as in adults. The composition of the fat in man as well as in different individuals of the same species of animals is rather variable, a fact which is probably dependent upon the food. According to the researches of HENRIQUES and HANSEN the fat of the subcutaneous fatty tissue is richer in olein than that of the internal organs; this has also been observed by LEICK and WINKLER.[4] In animals with a thick subcutaneous fat deposit the outer layers, according to HENRIQUES and HANSEN, are richer in olein than the inner layers. The fat of cold-blooded animals is especially rich in olein. The fat of domestic animals has, according to

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[1] Annal. d. Chem. u. Pharm., **142**.

[2] Ludwig-Festschrift, 1874, Leipzig.

[3] See Jaeckle, Zeitschr. f. physiol. Chem., **36** (literature).

[4] Knöpfelmacher, Jahrbuch f. Kinderheilkunde (N. F.), **45** (older literature); Siegert, Hofmeister's Beiträge, **1**; Jaeckle, Zeitschr. f. physiol. Chem., **36** (literature); Henriques and Hansen, Skand. Arch. f. Physiol., **11**; Leick and Winkler, Arch. f. Path. u. Pharm., 48.

AMTHOR and ZINK, a less oily consistency and a lower iodine and acetyl equivalent than the corresponding fat of wild animals. Under pathological conditions the fat may have a markedly pronounced variation. The fat of lipoma seems, according to JAECKLE, to be poorer in lecithin than other fats.

The properties of fats in general, and the three most important varieties of fat, have already been considered in a previous chapter, hence the formation of the adipose tissue is of chief interest at this time.

The formation of fat in the organism may occur in various ways. The fat of the animal body may consist partly of fat absorbed from the food and deposited in the tissues, and partly of fat formed in the organism from other bodies, such as proteins or carbohydrates.

That the fat from the food which is absorbed in the intestinal canal may be retained by the tissues has been shown in several ways. RADZIEJEWSKI, LEBEDEFF, and MUNK have fed dogs with various fats, such as linseed-oil, mutton-tallow, and rape-seed-oil, and have afterwards found the administered fat in the tissues. HOFMANN starved dogs until they appeared to have lost their fat and then fed them upon large quantities of fat and only little proteins. When the animals were killed, he found so large a quantity of fat that it could not have been formed from the administered proteins alone, but the greater part must have been derived from the fat of the food. PETTENKOFER and VOIT arrived at similar results in regard to the behavior of the absorbed fats in the organism, though their experiments were of another kind. MUNK has found that on feeding with free fatty acids, these are deposited in the tissues, not, however, as such; but they are transformed by synthesis with glycerine into neutral fats on their passage from the intestine into the thoracic duct. The connection between the fat of the food and of the body has also been shown by others, especially by ROSENFELD. CORONEDI and MARCHETTI and especially WINTERNITZ[1] have recently shown that the iodized fat is taken up in the intestinal tract and deposited in the various organs.

Proteins and carbohydrates are considered as the mother-substances of the fats formed in the organism.

The formation of the so-called *corpse-wax, adipocere,* which consists of a mixture of fatty acids, ammonia, and lime-soaps, from parts of the corpse rich in proteins, is sometimes given as a proof of the *formation of fats from proteins.* The accuracy of this view has, however, been disputed, and many other explanations of the formation of this substance have been offered. According to the experiments of KRATTER and K. B. LEHMANN, it seems as if it were possible by experimental means to convert animal tissue rich in proteins (muscles) into adipocere by the continuous action of water. Irrespective of this, **Salkowski** has shown recently that in the formation of adipocere the fat itself takes part, in that the olein decomposes with the formation of solid fatty acids; still it must be considered that lower organisms undoubtedly take part in its formation. The production of adipocere as a proof of the formation of fat from proteins is disputed by many investigators for this and other reasons.

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[1] Coronedi and Marchetti, cited by Winternitz, Zeitschr. f. physiol. Chem., **24**. A review of the literature on fat formation may be found in Rosenfeld, Fettbildung, in Ergebnisse der Physiologie, **1**, Abt. 1.

Fatty degeneration has been considered as another proof of the formation of fat from proteins. From the investigations of **Bauer** on dogs and **Leo** on frogs it was assumed that, at least in acute poisoning by phosphorus, a fatty degeneration, with the formation of fat from proteins, takes place. **Pflüger** has raised such strong arguments against the older researches as well as the more recent one of **Polimanti**, who claims to have shown the formation of fat from proteins in phosphorus poisoning, that we cannot consider the formation of fat as conclusively proved. Recent investigations of **Athanasiu**, **Taylor**, **Schwalbe**, and others, especially of **Rosenfeld**,[1] have made it probable that in these instances no new formation of fat from protein took place, but rather a fat migration (**Rosenfeld**).

Another more direct proof for the formation of fat from proteins has been given by **Hofmann**. He experimented with fly-maggots. A number of these were killed and the quantity of fat determined. The remainder were allowed to develop in blood whose proportion of fat had been previously determined, and after a certain time they were killed and analyzed. He found in them from seven to eleven times as much fat as was contained in the maggots first analyzed and the blood taken together. **Pflüger**[2] has made the objection that a considerable number of lower fungi develop in the blood under these conditions, in whose cell-body fats and carbohydrates are formed from the different constituents of the blood and their decomposition products, and that these serve as food for the maggots.

As a more convincing proof of fat formation from proteins, the investigations of **Pettenkofer** and **Voit** are often quoted. These investigators fed dogs with large quantities of meat containing the least possible proportion of fat, and found all of the nitrogen in the excreta, but only a part of the carbon. As an explanation of these conditions it has been assumed that the protein of the organism splits into a nitrogenized and a non-nitrogenized part, the former changing into the nitrogenized final product, urea, and like products, and the latter, on the contrary, being retained in the organism as fat (PETTENKOFER and VOIT).

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[1] Bauer, Zeitschr. f. Biologie, **7**; Leo, Zeitschr. f. physiol. Chem., **9**; Polimanti, Pflüger's Arch., **70**; Pflüger, *ibid.*, **51** (literature on the formation of fat from protein) and **71**; Athanasiu, *ibid.*, **74**; Taylor, Journ. Exp. Medicine, **4**; see also foot-note **1**, p. 283.

[2] See Rosenfeld, Fettbildung, Ergebnisse der Physiologie, **1**, Abt. **1**.

PFLÜGER has arrived at the following conclusion by an exhaustive criticism of PETTENKOFER and VOIT's experiments and a careful recalculation of their balance-sheet: that these very meritorious investigations, which were continued for a series of years, were subject to such great defects that they are not conclusive as to the formation of fat from proteins. He especially emphasizes the fact that these investigators started from a wrong assumption as to the elementary composition of the meat, and that the quantity of nitrogen assumed by them was too low and the quantity of carbon too high. The relationship of nitrogen to carbon in meat poor in fat was assumed by VOIT to be as 1: 3.68, while according to PFLÜGER it is 1:3.22 for fat-free meat after deducting the glycogen, and according to RUBNER 1.3.28 without deducting the glycogen. On recalculation of the figures using these coefficients, PFLÜGER has arrived at the conclusion that the assumption as to the formation of fat from proteins finds no support in these experiments.

In opposition to these objections, E. VOIT and M. CREMER have made new feeding experiments to show the formation of fat from proteins, but the proof of these recent investigations has been denied by PFLÜGER. On feeding a dog on meat poor in fat (containing a known quantity of ether extractives, glycogen, nitrogen, water, and ash), KUMAGAWA[2] could not prove the formation of fat from protein. According to him the animal body under normal conditions has not the power of forming fat from protein.

Several French investigators, especially CHAUVEAU, GAUTIER, and KAUFMANN, consider the formation of fat from proteins as positively proved. KAUFMANN has recently substantiated this view by a method which will be spoken of in detail in Chapter XVIII, in which he studied the nitrogen elimination and the respiratory gas exchange in conjunction with the simultaneous formation of heat.

As we are agreed that carbohydrates and glycogen, as well as sugar, can be formed from proteins, the fact cannot be denied that possibly an indirect formation of fat from proteins, with a carbohydrate as an intermediate step, can take place. The possibility of a direct fat formation from proteins without the carbohydrate as intermediary must also be generally admitted, although such a formation has not been conclusively proved.

According to CHAUVEAU and KAUFMANN, in the direct formation of fat

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[1] See Rosenfeld, Fettbildung, Ergebnisse der Physiologie. **1**, Abt. 1.

[2] Kaufmann, Arch. de physiol. (5) 8, where the works of Chauveau and Gautier are cited.

from proteins the fat is formed, besides urea, carbon dioxide, and water, as an intermediary product in the oxidation of the proteins, while GAUTIER considers the formation of fat from proteins as a cleavage without the taking up of oxygen. If fat is formed from protein in the animal body, then such formation is not a splitting off of fat from the proteins, but rather a synthesis from primarily formed cleavage products of proteins which are deficient in carbon.

The *formation of fat from carbohydrates* in the animal body was first suggested by LIEBIG. This was combated for some time, and until lately it was the general opinion that a direct formation of fat from carbohydrates not only had not been proved, but also that it was improbable. The undoubtedly great influence of the carbohydrates on the formation of fat as observed and proved by LIEBIG was explained by the statement that the carbohydrates were consumed instead of the absorbed fat or that derived from the proteins, hence they have a sparing action on the fat. By means of a series of nutrition experiments with foods especially rich in carbohydrates LAWES and GILBERT, SOXHLET, TSCHERWINSKY, MEISSL and STROMER (on pigs), B. SCHULTZE, CHANIEWSKI, E. VOIT and C. LEHMANN (on geese), I. MUNK and RUBNER and LUMMERT[1] (on dogs) apparently prove that a direct formation of fat from carbohydrates does actually occur. The processes by which this formation takes place are still unknown. As the carbohydrates do not contain as complicated carbon chains as the fats, the formation of fat from carbohydrates must consist of a synthesis, in which the group CHOH is converted into CH_2; hence a reduction must occur.

Analogous to NENCKI'S view as to the butyric-acid fermentation, when lactic acid is formed from the sugar and from this CO_2H_2 and acetaldehyde (C_2H_4O) are produced, and from this latter, by the union of two molecules, butyric acid is formed, so MAGNUS-LEVY[2] attempts to explain the formation of fat in the animal body from carbohydrates by synthesis from aldehyde and reduction. He considers that the process proceeds in the following way: (*a*) $9C_3H_6O_3 = 9C_2H_4O + 9H_2 + 9CO_2$ and (*b*) $9C_2H_4O + 7H_2 = C_{18}H_{36}O_2$ (stearic acid) $+ 7H_2O$.

After feeding with very large quantities of carbohydrates the relationship between the inspired oxygen and the expired carbon dioxide, i.e., the respiratory quotient $\frac{CO_2}{O}$, was found greater than 1 in certain cases (HAN-

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[1] Lawes and Gilbert, Phil. Transactions, 1859, part 2; Soxhlet, see Maly's Jahresber., **11**, 51; Tscherwinsky, Landwirthsch. Versuchsstaat, **29** (cited from Maly's Jahresber., **13**); Meissl and Stromer, Wien. Sitzungsber., 88, Abt. 3; Schultze, Maly's Jahresber., **11**, 47; Chaniewski, Zeitschr f. Biologie, **20**; Voit and Lehmann, see C. v Voit, Sitzungsber. d. k. bayer Akad. d. Wissensch., 1885; I. Munk, Virchow's Arch., **101**; Rubner, Zeitschr f. Biologie, **22**; Lummert, Pflüger's Arch., **71**.

[2] Arch. f. (Anat. u.) Physiol., 1901.

RIOT and RICHET, BLEIBTREU, KAUFMANN, LAULANIÉ [1]). This is explained by the assumption that the fat is formed from the carbohydrate by a cleavage setting free carbon dioxide and water without taking up oxgen. This increase in the respiratory quotient also depends in part on the increased combustion of the carbohydrate.

When food contains an excess of fat the superfluous amount is stored up in the fatty tissue, and on partaking of food deficient in fat this accumulation is quickly exhausted; and it is very probable that the lipase is of importance here, as LOEVENHART[2] has found that all over the body where fat is deposited in large amounts lipase also occurs in considerable amounts. There is perhaps not one of the various tissues that decreases so much in starvation as the fatty tissue. The organism, then, possesses in this tissue a depot where there is stored during proper alimentation a nutritive substance of great importance in the development of heat and vital force, which substance, on insufficient nutrition, is given up as may be needed. On account of their low conducting power, the fatty tissues become of great importance in regulating the loss of heat from the body. They also serve to fill cavities and act as a protection and support to certain internal organs.

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[1] Hanriot and Richet, Annal. de Chim. et de Phys. (6), 22; Bleibtreu, Pflüger's Arch., 56 and 85; Kaufmann, Arch. de Physiol. (5), 8; Laulanié, *ibid.*, 791.

[2] Amer. Journ. of Physiol., 6.

CHAPTER XI.

MUSCLES.

Striated Muscles.

IN the study of the muscles the chief problem for physiological chemistry is to isolate their different morphological elements and to investigate each element separately. By reason of the complicated structure of the muscles this has been thus far almost impossible, and we must be satisfied at the present time with a few microchemical reactions in the investigation of the chemical composition of the muscular fibres.

Each muscle-tube and each muscle-fibre consists of a sheath, the SARCOLEMMA, which seems to be composed of a substance similar to elastin, and containing a large proportion of *protein*. This last, which in life possesses the power of contractility, has in the inactive muscle an alkaline reaction, or, more correctly speaking, an amphoteric reaction with a pre dominating action on red litmus paper. RÖHMANN has found that the fresh, inactive muscle shows an alkaline reaction with red lacmoid, and an acid reaction with brown turmeric. From the behavior of these coloring-matters with various acids and salts he concludes that the alkalinity of the fresh muscle with lacmoid is due to sodium bicarbonate, diphosphate, and probably also to an alkaline combination of protein bodies, and the acid reaction with turmeric, on the contrary, to monophosphate chiefly. The dead muscle has an acid reaction, or, more correctly, the acidity with turmeric increases on the decease of the muscle, and the alkalinity with lacmoid decreases. The difference depends on the presence of a larger quantity of monophosphate in the dead muscle, and according to RÖHMANN free lactic acid is found in neither the one case nor the other.[1]

If the somewhat disputed statements relative to the finer structure of the muscles are disregarded, one can differentiate in the striated muscles between the two chief components, the doubly refracting—*anisotropous*—and the singly refracting—*isotropous*—substance. If the muscular fibres are treated with reagents which dissolve proteins, such as dilute hydrochloric acid, soda solution, or gastric juice, they swell greatly and break up into "BOWMAN'S *disks.*" By the action of alcohol, chromic acid, boiling water, or in general such reagents as cause a shrinking, the fibres split longitudinally into fibrils; and this behavior shows that several chemically different substances of various solubilities enter into the construction of the muscular fibres.

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[1] The various statements in regard to the reaction of the muscles and the cause thereof are conflicting. See Röhmann, Pflüger's Arch., 50 and 55; Heffter, Arch. f. exp. Path. u. Pharm., 31 and 38. These references contain the pertinent literature.

The protein myosin is generally considered as the chief constituent of the diagonal disks, while the isotropous substance contains the chief mass of the other proteins of the muscles as well as the chief portion of the extractives. According to the observations of DANILEWSKY, confirmed by J. HOLMGREN,[1] myosin may be completely extracted from the muscle without changing its structure, by means of a 5 per cent solution of ammonium chloride, which fact contradicts the above view. DANILEWSKY claims that another protein-like substance, insoluble in ammonium chloride and only swelling up therein, enters essentially into the structure of the muscles. The proteins, which form the chief part of the solids of the muscles, are of the greatest importance.

Proteins of the Muscles.

Like the blood which contains a fluid, the blood-plasma, which spontaneously coagulates, separating fibrin and yielding blood-serum, so also the living muscle, at least of cold-blooded animals, contains, as first shown by KÜHNE, a spontaneously coagulating liquid, the muscle-plasma, which coagulates quickly, separating a protein body, myosin, and yielding also a serum. That liquid which is obtained by pressing the living muscle is called *muscle-plasma,* while that obtained from the dead muscle is called *muscle-serum.* These two fluids contain different protein bodies.

Muscle-plasma was first prepared by KÜHNE from frog-muscles, and later by HALLIBURTON, according to the same method, from the muscles of warm-blooded animals, especially rabbits. The principle of this method is as follows: The blood is removed from the muscles immediately after the death of the animal by passing through them a strongly cooled common-salt solution of 5–6 p. m. Then the muscles are quickly cut and immediately thoroughly frozen so that they can be ground in this state to a fine mass— "muscle-snow." This pulp is strongly pressed in the cold, and the liquid which exudes is called muscle-plasma. According to v. FÜRTH [2] this cooling or freezing is not necessary. It is sufficient to extract the muscle free from blood, as above directed, with a 6 p. m. common-salt solution.

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[1] Danilewsky, Zeitschr. f. physiol. Chem., **7**; J. Holmgren, Maly's Jahresber., **23**.

[2] See Kühne, Untersuchungen über das Protoplasma (Leipzig, 1864), 2; Halliburton, Journ. of Physiol., 8; v. Fürth, Arch. f. exp. Path. u. Pharm., **36** and **37**; Hofmeister's Beiträge, **3**, and Ergebnisse der Physiologie, **1**, Abt. **1**; Stewart and Sollmann, Journ. of Physiol., **24**.

Muscle-plasma forms a yellow to brownish-colored fluid with an alkaline reaction. It is somewhat different in different animals. Muscle-plasma from the frog spontaneously coagulates slowly at a little above 0° C., but more quickly at the temperature of the body. Muscle-plasma from mammals coagulates slowly, according to v. FÜRTH, even at the temperature of the room, though only slightly, and it can hardly be considered as a process comparable with the coagulation of the blood. Indeed the question may be asked whether a true muscle-plasma does exist in warm-blooded animals, or whether the fluid obtained from such muscles exactly represents the plasma of the living muscle. According to KÜHNE and v. FÜRTH the reaction remains alkaline during coagulation, while according to HALLIBURTON, STEWART and SOLLMANN, it becomes acid. According to the older views the clot consists of a globulin called myosin, while v. FÜRTH claims that it consists of two coagulated proteins, myosin-fibrin and myogen-fibrin.

The study of the proteins of the muscles, as well as their nomenclature, has changed markedly in the last few years, and it is questionable whether an essential difference exists between the proteins of the muscle-plasma and the muscle-serum of warm-blooded animals. Nevertheless it is necessary to separately discuss the proteins of the dead muscle as well as those of the muscle-plasma.

The *proteins of the dead muscle* are in part soluble in water or dilute salt solutions, and in part are insoluble therein. Myosin and musculin and also myoglobulin and myoälbumin, which exist to a very slight extent and are perhaps only derived from the remaining lymph, belong to the first group, and the stroma substances of the muscle-tubes belong to the second group.

Myosin was first discovered by KÜHNE, and constitutes the principal mass of the soluble proteins of the dead muscle. It is generally considered as the most essential coagulation product of muscle-plasma. The name myosin KÜHNE also gives to the mother-substance of the plasma-clot, and this mother-substance forms, according to certain investigators, the chief mass of contractile protoplasm. The statements as to the occurrence of myosin in other organs besides the muscles require further proof. The quantity of myosin in the muscles of different animals varies, according to DANILEWSKY,[1] between 30 and 110 p. m.

Myosin, as obtained from dead muscles, is a globulin whose elementary composition, according to CHITTENDEN and CUMMINS,[2] is, on an average, the following: C 52.28, H 7.11, N 16.77, S 1.27, O 22.03 per cent. If the myosin separates as fibres, or if a myosin solution with a minimum quantity of alkali is allowed to evaporate on a microscope-slide to a gelatinous mass, doubly refracting myosin may be obtained. Myosin has the general properties of the globulins. It is insoluble in water, but soluble in dilute saline solutions as well as in dilute acids or alkalies, which readily convert it into albuminates. It is completely precipitated upon saturation with NaCl, also by $MgSO_4$, in a solution containing 94 per cent of the salt with its water of crystallization (HALLIBURTON). The precipitated myosin becomes insoluble readily. Like fibrinogen it coagulates at 56° C. in a solution containing common salt, but differs from it since under no circumstances can it be converted into fibrin. The coagulation temperature, according to CHITTENDEN and CUMMINS, not only varies for myosins of different origin, but also for the same myosin in different salt solutions.

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[1] Zeitschr. f. physiol. Chem., 7.

[2] Studies from the Physiol. Chem. Laboratory of Yale College, New Haven, 3, 115.

Myosin may be prepared in the following way, as suggested by HALLIBURTON: The muscle is first extracted by a 5 per cent magnesium-sulphate solution. The filtered extract is then treated with magnesium sulphate in substance until 100 c.c. of the liquid contains about 50 grams of the salt. The so-called paramyosinogen or musculin separates. The filtered liquid is then treated with magnesium sulphate until each 100 c.c. of the liquid holds 94 grams of the salt in solution. The myosin which now separates is filtered off, dissolved in water by aid of the retained salt, precipitated by diluting with water, and, when necessary, purified by redissolving in dilute salt solution and precipitating with water.

The older and perhaps the usual method of preparation consists, according to DANILEWSKY,[1] in extracting the muscle with a 5–10 per cent ammonium-chloride solution, precipitating the myosin from the filtrate by strongly diluting with water, and redissolving the precipitate in ammonium-chloride solution, and the myosin obtained from this solution is reprecipitated either by diluting with water or by removing the salt by dialysis.

Musculin,[2] called PARAMYOSINOGEN by HALLIBURTON, and MYOSIN by v. FÜRTH, is a globulin which is characterized by its low coagulation temperature, about 47° C., which may vary in different species of animals (45° in frogs, 51° C. in birds). It is more easily precipitated than myosin by NaCl or $MgSO_4$ (50 per cent salt, including water of crystallization). According to v. FÜRTH it is precipitated by ammonium sulphate with a concentration of 12–24 per cent salt. If the dead muscle is extracted with water a part of the musculin goes into solution and may be precipitated therefrom by carefully acidifying. It separates from a dilute salt solution on dialysis. Musculin readily passes into an insoluble modification which

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[1] Zeitschr. f. physiol. Chem., 5, 158.

[2] As we have up to the present no conclusive basis for the identity of the globulins called myosin and paramyosinogen, and also as the use of the name myosin for the last-mentioned substance may readily cause confusion, the author does not feel justified in dropping the old name musculin (Nasse).

v. FÜRTH calls *myosin fibrin.* Musculin is called myosin by v. FÜRTH, as he considers it nothing but myosin. As musculin has a lower coagulation temperature and has other precipitating properties for neutral salts than the older substance called myosin, it is difficult to concede to this view.

Myoglobulin. After the separation of the musculin and the myosin from the salt extract of the muscle by means of $MgSO_4$ the myoglobulin may be precipitated by saturating the filtrate with the salt. It is similar to serglobulin, but coagulates at 63° C. (HALLIBURTON). *Myoalbumin,* or muscle-albumin, seems to be identical with seralbumin (seralbumin α, according to HALLIBURTON), and probably originates only from the blood or the lymph. Proteoses and peptones do not seem to exist in the fresh muscles

After the complete removal from the muscle of all protein bodies which are soluble in water and ammonium chloride, an insoluble protein remains which only swells in ammonium-chloride solution, and which forms with the other insoluble constituents of the muscular fibre the "*muscle-stroma.*" According to DANILEWSKY the amount of such stroma substance is connected with the muscle activity. He maintains that the muscles contain a greater amount of this substance, compared with the myosin present, when the muscles are quickly contracted and relaxed.

According to J. HOLMGREN,[1] this stroma substance does not belong to either the nucleoalbumin or the nucleoproteid group. It is not a glucoproteid, as it does not yield a reducing substance when boiled with dilute mineral acids. It is very similar to the coagulable proteins and dissolves in dilute alkalies, forming an albuminate. The elementary composition of this substance is nearly the same as that of myosin. There is no doubt that the insoluble substances, myofibrin and myosin fibrin, which are formed, according to v. FÜRTH, in the coagulation of the plasma, occur also among the stroma substances. When the muscles are previously extracted with water the stroma substance also contains a part of the myosin hereby made insoluble. To the proteins insoluble in water and neutral salts belongs the *nucleoproteid* detected by PEKELHARING, which occurs as traces and is soluble in faintly alkaline water, and which originates probably from the muscle nuclei. According to BOTTAZZI and DUCCESCHI[2] the heart muscle is richer in nucleoproteid than the skeletal muscle.

Muscle-syntonin, which may be obtained by extracting the muscles with hydrochloric acid of 1 p. m., and which, according to K. MÖRNER, is less soluble and has a greater aptitude to precipitate than other acid albumins, seems not to occur preformed in the muscles. HEUBNER'S[3] *mytolin* is modified muscle-proteid, chiefly myosin, which has lost a part of its sulphur by the action of alkali.

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[1] See foot-note 1, p. 448.

[2] Pekelharing, Zeitschr. f. physiol. Chem., 22; Bottazzi and Ducceschi, Centralbl. f. Physiol., 12.

[3] Arch. f. exp. Pathol. u. Pharm., 53.

Proteins of the Muscle-plasma. As above stated, myosin was ordinarily considered as the coagulated modification of a soluble protein existing in the muscle-plasma. As in blood-plasma there is present a mother-substance of fibrin, fibrinogen, so also there exists in the muscle-plasma a mother-substance of myosin, a soluble myosin or a *myosinogen.* This body has not thus far been isolated with certainty. HALLIBURTON, who has detected in the muscles an enzyme-like substance, "*myosin ferment,*" which is related to fibrin ferment but not identical with it, has also found that a solution of purified myosin, in dilute salt solution (5 per cent $MgSO_4$), and sufficiently diluted with water, coagulates after a certain time, and at the same time becomes acid, and a typical myosin-clot separates. This coagulation, which is accelerated by warming or by the addition of myosin ferment, is, according to HALLIBURTON, a process analogous to the coagulation of the muscle-plasma. According to this same investigator, myosin when dissolved in water by the aid of a neutral salt is reconverted into myosinogen, while after diluting with water myosin is again produced from the myosinogen. The musculin (paramyosinogen) is carried down, according to HALLIBURTON, with the myosin-clot, but has nothing to do with the coagulation, as the myosin-clot forms also in the absence of musculin, and this last is not changed into myosin.

Besides the traces of globulin and albumin, which perhaps do not belong to the muscle-plasma, there occur in mammals, according to v. FÜRTH, two proteins, namely, musculin (myosin according to v. FÜRTH) and myogen.

MUSCULIN (NASSE) = paramyosinogen (HALLIBURTON) = myosin (v. FÜRTH) forms about 20 per cent of the total proteins of the muscle-plasma of rabbits. Its properties have already been given, and it is sufficient to remark that its solutions become cloudy on standing, and a precipitate of *myosin fibrin* occurs, which is insoluble in salt solutions.

Myogen, or MYOSINOGEN (HALLIBURTON), forms the chief mass, 75–80 per cent, of the proteins of rabbit muscle-plasma. It does not separate from its solutions on dialysis and is not a true globulin, but a protein *sui generis.* It coagulates at 55–65° C. and is precipitated in the presence of 26–40 per cent ammonium sulphate. Myogen solutions are precipitated by acetic acid only in the presence of some salt. It is converted into an albuminate by alkalies, this albuminate being precipitable by ammonium chloride. Myogen passes spontaneously, especially with higher temperatures as well as in the presence of salt, into an insoluble modification, *myogen fibrin.* A protein, coagulating at 30–40° C., *soluble myogen fibrin,* is produced as a soluble intermediate step. This substance occurs to a considerable extent in native frog-muscle plasma. It does not always occur in the muscle-plasma of warm-blooded animals, and when it does it is present only to a slight extent. It can be separated by precipitating with salt or by diffusion. HALLIBURTON'S assumption as to the action of a special myosin ferment has not sufficient basis, according to v. FÜRTH, nor has the often-admitted analogy with the coagulation of the blood. The difference between the musculin and the myogen in their becoming insoluble is that the musculin passes into myosin fibrin without any soluble intermediate steps.

Myogen may be prepared, according to v. FÜRTH, by heating for a short time the dialyzed and filtered plasma to 52° C., separating it in this way from the rest of the musculin. The myogen exists in the new filtrate and can be precipitated by ammonium sulphate. The musculin may also be removed by adding 28 per cent ammonium sulphate and then precipitating the myogen from the filtrate by saturating with the salt.

STEWART and SOLLMANN admit of only two soluble proteins in the muscles. One is the paramyosinogen, which is the same as v. FÜRTH's myosin + the soluble myogen fibrin. The other they call myosinogen, which corresponds to v. FÜRTH's myogen or to HALLIBURTON's myosinogen + myoglobulin. It is an atypical globulin which coagulates at 50–60° C. The paramyosinogen as well as the myosinogen are readily converted into an insoluble modification, myosin. The myosin of the above investigators is the same as v. FÜRTH's myosin fibrin + myogen fibrin, and corresponds, it seems, also to myosin mixed with paramyosinogen (HALLIBURTON). STEWART and SOLLMANN differ from HALLIBURTON in considering that paramyosinogen also coagulates and is converted into myosin. According to them myosin is also insoluble in a NaCl solution.

The views of the various investigators differ so essentially and the nomenclature is so complicated (four different things are designated by the name myosin) that it is extremely difficult to give any correct review of the various notions.[1] Thorough investigations on this subject are very necessary.

Myoproteid is a proteid found by v. FÜRTH in the plasma from fish-muscles. It does not coagulate on boiling, is precipitated by acetic acid, and considered as a compound proteid by v. FÜRTH.

In connection with v. FÜRTH's work, PRZIBRAM has carried on investigations on the occurrence of muscle-proteins in various classes of animals. The myosin (v. FÜRTH) and myogen occur in all classes of vertebrates; the myogen is always absent in the invertebrates. Myoproteid occurs, at least in considerable quantity, only in fishes. In the muscle after cutting the nerve, STEYRER[2] found somewhat more musculin and less myogen in the muscle-juice than in the normal muscle.

Muscle-pigments. There is no question that the red color of the muscles, even when completely freed from blood depends in part on hæmoglobin.

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[1] For these reasons the author is not sure whether he has understood and correctly given the work of the different investigators.

[2] Przibram, Hofmeister's Beiträge, 2; Steyrer, *ibid.*, 4.

K. MÖRNER has shown that muscle-hæmoglobin is not quite identical with blood-hæmoglobin. The statement of MACMUNN, that in the muscles another pigment occurs which is allied to hæmochromogen and called *myohæmatin* by him, has not been substantiated, at least for muscles of higher animals (LEVY and MÖRNER[1]). MACMUNN claims that myohæmatin occurs in the muscles of insects, which do not contain any hæmoglobin. The reddish-yellow coloring-matter of the muscles of the salmon has been little studied.

Various enzymes have been found in the muscles. To these belong (besides traces of fibrin ferment and myosin ferment) the *catalases* and *oxidases*, which occur only to a slight extent. The disputed glycolytic enzyme (Chapter VIII), whose nature is unknown, probably belongs to the oxidases. An amylolytic and a proteolytic enzyme (HEDIN and ROWLAND[2]) have also been found, and the hydrolytic and oxidizing enzymes (Chapter XV) active in the formation and destruction of uric acid are also present.

Extractive Bodies of the Muscles.

The nitrogenous extractives consist chiefly of *creatine*, on an average of 1–4 p. m. in the fresh muscles containing water, also the *purine bases*, *hypoxanthine* and *xanthine*, besides *guanine* and *carnine*, but chiefly hypoxanthine. The purine bases probably do not occur as such but as complex combinations. The quantity of nitrogen as purine bases amounts, according to BURIAN and HALL, in the fresh flesh of the horse, ox, and calf to 0.55, 0.63, and 0.71 p. m. respectively, or 1.3–1.7 p. m., calculated as hypoxanthine. In the embryonic ox-muscles, KOSSEL[3] found more guanine than hypoxanthine. The purine bases are produced in the muscles themselves, and their production, which also takes place while at rest, is greatly increased during work (BURIAN[4]).

Among the apparently habitually occurring nitrogeneous extractives, we should also mention *phosphocarnic acid* as well as *inosinic acid*, which is perhaps allied to it, *carnosine*, *carnitine*, and perhaps also other bodies which have recently been found in meat extract and which will be mentioned later.

Among the extractive substances is also found the acid noticed by LIMPRICHT in the flesh of certain cyprinidea, namely, the nitrogenized *protic acid*, while the *isocreatinine* found by J. THESEN in fish-flesh is nothing but impure creatinine, according to Poulsson, Schmidt and Korndörfer.[1] *Uric acid, urea, taurine,* and *leucine* are found as traces in the muscles, in certain cases only in a few species of animals. In regard to the amounts of these different extractives in the muscles, Krukenberg and Wagner[2] have shown that it varies greatly in different animals A large quantity of urea is found in the muscles of the shark and ray; uric acid is found in alligators; taurine in cephalopoda; *glycocoll* in gasteropoda, and *creatinine* especially in fishes. The reports are very contradictory in regard to the occurrence of urea in the muscles of higher animals. According to the investigations of Kaufmann and Schondörff, confirmed by Brunton-Blaikie,[3] urea is a regular constituent of the muscles, although M. Nencki and Kowarski dispute this.

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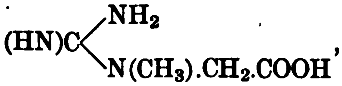
[1] See MacMunn, Phil. Trans. of Roy. Soc., **177**, part 1, Journ. of Physiol., **8**, and Zeitschr. f. physiol. Chem., **13**; Levy, *ibid.*, **13**; K. Mörner, Nord. Med. Archiv, Festband, 1897, and Maly's Jahresber., **27**.

[2] Zeitschr. f. physiol. Chem., **32**.

[3] Burian and Hall, Zeitschr. f. physiol. Chem., **38**; Kossel, *ibid.*, **8**, **408**.

[4] *Ibid.*, **43**.

The xanthine bodies, with the exception of carnine, have been treated on pages 159–162, and therefore among the extractive bodies we will first consider the creatine.



Creatine, $C_4H_9N_3O_2$, $(HN)C\begin{cases}NH_2\\N(CH_3).CH_2.COOH\end{cases}$, or methylguanidine-acetic acid, occurs in the muscles of vertebrate animals in variable amounts in different species; the largest quantity is found in birds. It is also found in the brain, blood, transudates, amniotic fluid, and sometimes also in the urine. Creatine may be prepared synthetically from cyanamide and sarcosine (methylglycocoll). On boiling with baryta water it decomposes with the addition of water and yields urea, sarcosine, and certain other products. Because of this behavior several investigators consider creatine as a step in the formation of urea in the organism. On boiling with acids, creatine is easily converted, with the elimination of water into creatinine, $C_4H_7N_3O$, which occurs in urine, and which has also been found in the muscles of the dog by Monari[4] (see Chapter XV), and probably is a regular constituent of the muscles.

Creatine crystallizes in hard, colorless, monoclinic prisms which lose their water of crystallization at 100° C. It is soluble in 74 parts of water at the ordinary temperature and 9419 parts absolute alcohol. It dissolves more easily with the aid of heat. Its watery solution has a neutral reaction. Creatine is not dissolved by ether. If a creatine solution is boiled with precipitated mercuric oxide, this is reduced, especially in the presence of alkali, to mercury and oxalic acid, and the foul-smelling methyluramine (methylguanidine) is developed. A solution of creatine in water is not precipitated by basic lead acetate, but gives a white, flaky precipitate with mercurous nitrate if the acid reaction is neutralized. When boiled for an hour with dilute hydrochloric acid creatine is converted into creatinine and may be identified by its reactions. On boiling with formaldehyde it can be transformed into dioxymethylenecreatinine, which crystallizes readily (JAFFÉ[1]).

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[1] See Limpricht, Annal. d. Chem. u. Pharm., **127**, and Thesen, Zeitschr. f. physiol, Chem., **24**; Poulsson, Arch. f. exp. Path. u. Pharm., **51**; Schmidt and Korndörfer. *ibid.*, **51**.

[2] Zeitschr. f. Biologie, **21**; see also M. Henze, Zeitschr. f. physiol. Chem., **43**; Mendel, Hofmeister's Beiträge, **5**; Kelly, *ibid.*, 5.

[3] Kaufmann, Arch. de Physiol. (5), **6**; Schöndorff, Pflüger's Arch., **62**; Nencki and Kowarski, Arch. f. exp. Path. u. Pharm., **36**; Brunton-Blaikie, Journ. of Physiol., **23**, Supplement.

[4] Maly's Jahresber., **19**, 296.

The preparation and detection of creatine is best performed by the following method of NEUBAUER,[2] which was first used in the preparation of creatine from muscles: Finely cut meat is extracted with an equal weight of water at 50° to 55° C. for 10–15 minutes, pressed, and extracted again with water. The proteins are removed from the united extracts as far as possible by coagulation at boiling heat, the filtrate precipitated by the careful addition of basic lead acetate, the lead removed from this filtrate by H_2S and the solution then carefully concentrated to a small volume. The creatine, which crystallizes in a few days, is collected on a filter, washed with alcohol of 88 per cent, and purified, when necessary, by recrystallization. The quantitative estimation of creatine is performed according to the same method.

Carnine, $C_7H_8N_4O_3+H_2O$, is one of the substances found by WEIDEL in American meat extract. It has also been found by KRUKENBERG and WAGNER in frog-muscles and in the flesh of fishes, and by POUCHET[3] in the urine. Carnine may be transformed into hypoxanthine by oxidation.

Carnine has been obtained as a white crystalline mass. It dissolves with difficulty in cold water, but more readily in warm. It is insoluble in alcohol and ether. It dissolves in warm hydrochloric acid and yields a salt crystallizing in shining needles, which gives a double compound with platinum chloride. Its watery solution is precipitated by silver nitrate, but this precipitate is dissolved neither by ammonia nor by warm nitric acid. Carnine does not give the so-called WEIDEL's xanthine reaction. Its watery solution is precipitated by basic lead acetate; but the lead compound may be dissolved on boiling.

Carnine is prepared by the following method: The meat extract diluted with water is completely precipitated by baryta-water. The filtrate is precipitated by basic lead acetate, the lead precipitate boiled with water, filtered while hot, and sulphuretted hydrogen passed through the filtrate. Remove the lead sulphide from the filtrate and concentrate strongly. The concentrated solution is now completely precipitated with silver nitrate, the precipitate washed free from silver chloride by ammonia, and the carnine silver oxide suspended in water and treated with sulphuretted hydrogen.

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[1] Ber. d. d. chem. Gesellsch., **35**.

[2] Zeitschr. f. physiol. Chem., **2** and **6**.

[3] Weidel, Annal. d. Chem. u. Pharm., **158**; Krukenberg and Wagner, Sitzungsber. d. Würzb. phys.-med. Gesellsch., 1883; Pouchet, cited from Neubauer-Huppert, Analyse des Harnes, 10. Aufl., 335.

Carnosine, $C_9H_{14}N_4O_3$, has been isolated by Gulewitsch and Amiražibi[1] from meat extracts. It is a base which is perhaps related to arginine, and is readily soluble in water, crystallizing in flat needles. It is precipitated by phosphotungstic acid and by silver nitrate in the presence of an excess of barium hydrate and forms a copper compound which crystallizes in hexagonal plates.

Carnitine, $C_7H_{15}NO_3$ (?), is another base isolated by Gulewitsch and Krimberg[2] from meat extracts, has a strong alkaline reaction, and is very readily soluble in water. It gives a crystalline chloroplatinate, as well as salts with HCl and HNO_3 which are very readily soluble in water. The HNO_3 salt, which is also crystalline, is strongly levorotatory.

From Liebig's extract of beef Kutscher has recently isolated a series of new bodies, namely, *ignotine*, $C_9H_{14}N_4O_3$, *carnomuscarine*, *neosine*, $C_6H_{17}NO_2$, *novaine*, $C_7H_{17}NO_2$, *methylguanidine* (also found by Gulewitsch), and a crystallizable chloroplatinate, $C_{18}H_{38}N_2O_5.2HCl.PtCl_4$, of a body which he calls *oblitine*. Zunz[3] has also been able to isolate from fresh muscles the three hexone bases, leucine, aspartic acid, and glutamic acid. He has not decided whether these bodies exist preformed in the muscles.

The base *musculamine*, isolated by Etard and Vila on the hydrolysis of veal, is nothing but cadaverine, according to Posternak.[4]

Inosinic acid has been discussed on page 155. We must also include among the nitrogenous extractives those bodies which were first discovered by Gautier[5] and which occur only in very small quantities, namely, the leucomaines, *xanthocreatinine*, $C_5H_{10}N_4O$, *crusocreatinine*, $C_5H_8N_4O$, *amphicreatine*, $C_9H_{19}N_7O_4$, and *pseudoxanthine*, $C_4H_5N_5O$.

In the analysis of meat and for the detection and separation of the various extractive bodies of the same we make use of the systematic method as suggested by Gautier,[6] for details of which the reader is referred to the original article.

Phosphocarnic acid[7] is a complicated substance, first isolated by Siegfried from meat extracts, which yields as cleavage products succinic acid, paralactic acid, carbon dioxide, phosphoric acid, and a carbohydrate group, besides the previously mentioned carnic acid, which is identical with or nearly related to antipeptone. It stands, according to Siegfried, in close relationship to the nucleins, and as it yields peptone (carnic acid), it is designated as a *nucleon* by Siegfried. Phosphocarnic acid may be precipitated as an iron compound, *carniferrine*, from the extract of the muscles free from proteins. The quantity of phosphocarnic acid, calculated as carnic acid, can be determined by multiplying the quantity of nitrogen in the compound by the factor 6.1237 (Balke and Ide). In this way Siegfried found 0.57–2.4 p. m. carnic acid in the resting muscles of the dog, and M. Müller 1–2 p. m. in the muscles of adults and a maximum of 0.57 p. m. in those of new-born infants. According to Cavazzani nucleon occurs to a much greater extent in oysters, namely, an average of 3.725 p. m. It also occurs, as he and Manicardi found, in the plant kingdom. Phosphocarnic acid has not been prepared in the pure state and possesses on this account a variable composition; according to SIEGFRIED it serves as a source of energy in the muscles and is consumed during work. Besides, by means of its property of forming soluble salts with the alkaline earths, as also an iron combination soluble in alkalies, it acts as a means of transportation for these bodies in the animal body.

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[1] Zeitschr. f. physiol. Chem., **30**.

[2] *Ibid.*, 45.

[3] Kutscher, Zeitschr. f. Unters. d. Nahrungs- u. Genussmittel, **10**, and Centralbl. f. Physiol., **19**; Zunz, reference, *ibid.*, **18**; Gulewitsch, Zeitschr. f. physiol. Chem., **47**.

[4] Etard and Vila, Compt. rend., **135**; Posternak, *ibid.*, **135**.

[5] See Maly's Jahresber., **16**, 523.

[6] *Ibid.*, **22**, 335.

[7] In regard to carnic acid and phosphocarnic acid, see the works of Siegfried, Arch. f. (Anat. u.) Physiol., 1894, Ber. d. deutsch. chem. Gesellsch., **28**, and Zeitschr. f. physiol. Chem., **21** and **28**; M. Müller, *ibid.*, **22**; Krüger, *ibid.*, **22** and **28**; Balke and Ide, *ibid.*, **21**, and Balke, *ibid.*, **22**; Macleod, *ibid.*, **28**; E. Cavazzani, Centralbl. f. Physiol., **18**, 666; Panella, Maly's Jahresber., **34**.

Phosphocarnic acid is prepared from the extract free from protein by first removing the phosphate by $CaCl_2$ and NH_3. The acid is precipitated as carniferrine by ferric chloride from the filtrate while boiling.

The **non-nitrogenous extractive bodies** of the muscles are *inosite, glycogen, sugar,* and *lactic acid.*

Inosite, $C_6H_{12}O_6+H_2O=C_6H_6(OH)_6+H_2O$. This body, discovered by SCHERER, is not a carbohydrate, but a hexahydroxybenzene (MAQUENNE[1]). With hydriodic acid it yields benzene and tri-iodophenol. Inosite is found in the muscles, liver, spleen, leucocytes, kidneys, suprarenal capsule, lungs, brain, testicles, and in the urine in pathological cases, and as traces in normal urine. It is found very widely distributed in the vegetable kingdom, especially in the unripe fruit of green beans (Phaseolus vulgaris), and therefore it is also called PHASEOMANNITE. According to WINTERSTEIN a phosphorized compound occurs in the vegetable kingdom which yields inosite as a cleavage product. This compound is, according to POSTERNAK,[2] probably oxymethylphosphoric acid, which also yields inosite on decomposition by condensation.

Inosite crystallizes in large, colorless, rhombic crystals of the monoclinic system, or, if not pure and if only a small quantity crystallizes, it forms groups of fine crystals similar to cauliflower. It loses its water of crystallization at 110° C., also if exposed to the air for a long time. Such exposed crystals are non-transparent and milk-white. The crystals melt at 225° C. when dry. Inosite dissolves in 7.5 parts of water at ordinary temperature, and the solution has a sweetish taste. It is insoluble in strong alcohol and in ether. It dissolves cupric hydrate in alkaline solutions, but does not reduce on boiling. It gives negative results with MOORE'S test and with BÖTTGER-ALMÉN'S bismuth test. It does not ferment with beer-yeast, but may undergo lactic- and butyric-acid fermentation. The lactic acid formed thereby is sarcolactic acid according to HILGER, and fermentation lactic acid according to VOHL.[3] Inosite is oxidized into rhodizonic acid by an excess of nitric acid, and the following reactions depend upon this behavior:

If inosite is evaporated to dryness on platinum-foil with nitric acid and the residue treated with ammonia and a drop of calcium-chloride solution and carefully re-evaporated to dryness, a beautiful rose-red residue is obtained (SCHERER's inosite test). If we evaporate an inosite solution to incipient dryness and moisten the residue with a little mercuric nitrate solution, there is obtained a yellowish residue on drying, which becomes a beautiful red on strongly heating. The coloration disappears on cooling, but it reappears on gently warming (GALLOIS' inosite test).

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[1] Bull. de la soc. chim. (2), **47** and **48**; Compt. rend., **104**.

[2] Winterstein, Ber. d. d. chem. Gesellsch., **30**; Posternak, Contribution à l'étude chim. de l'assimilation chlorophyllienne, Revue générale de Botanique, **12** (1900).

[3] Hilger, Annal. d. Chem. u. Pharm., **160**; Vohl, Ber. d. d. chem. Gesellsch., **9**.

To prepare inosite from a liquid or from a watery extract of a tissue, the proteins are first removed by coagulation at boiling heat. The filtrate is precipitated by sugar of lead, this filtrate boiled with basic lead acetate and allowed to stand 24–48 hours. The precipitate thus obtained, which contains all the inosite, is decomposed in water by H_2S. The filtrate is strongly concentrated, treated with 2–4 vols. hot alcohol, and the liquid removed as soon as possible from the tough or flaky masses which ordinarily separate. If no crystals separate from the liquid within twenty-four hours, then treat with ether until the liquid has a milky appearance and allow it to stand. In the presence of a sufficient quantity of ether, crystals of inosite separate within twenty-four hours. The crystals thus obtained, as also those which are obtained from the alcoholic solution directly, are recrystallized by redissolving in very little boiling water and adding 2–4 vols. of alcohol.

Glycogen is a constant constituent of the living muscle, while it may be absent in the dead muscle. The quantity of glycogen varies in the different muscles of the same animal. BÖHM [1] found 10 p. m. glycogen in the muscles of cats, and moreover he found a greater amount in the muscles of the extremities than in those of the rump. SCHÖNDORFF has found a maximum of 37.2 p. m. in the dog-muscle. The statements as to the quantity of glycogen in the heart differ somewhat; although the heart is considered as somewhat poorer in glycogen than the other muscles, still this difference is not very great and can be explained by the ready disappearance of glycogen from the heart after death, as well as after starvation and after strong work (BORUTTAU, JENSEN, KISCH [2]). Work and food have a great influence upon the quantity of glycogen. BÖHM found 1–4 p. m. glycogen in the muscles of fasting animals, and 7–10 p. m. after partaking of food. As stated in Chapter VIII, work, starvation, and lack of carbohydrates in the food cause the glycogen to disappear earlier from the liver than from the muscles.

The *sugar of the muscles*, of which only traces occur in the living muscle, and which is probably formed after the death of the muscle from the muscle-glycogen, is, according to the investigations of PANORMOFF, in part dextrose, but consists chiefly of maltose (OSBORNE and ZOBEL [3]) with some dextrin.

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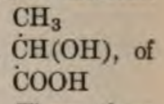
[1] Böhm, Pflüger's Arch., 23, 44; Schöndorff, *ibid.*, 99.

[2] Boruttau, Zeitschr. f. physiol. Chem., 18; Jensen, *ibid.*, 35; Kisch, Hofmeister's Beiträge, 8.

[3] Panormoff. Zeitschr. f. physiol. Chem., 17; Osborne and Zobel, Journ. of Physiol., 29.

Lactic Acids. Of the oxypropionic acids with the formula $C_3H_6O_3$ there is one, ethylene lactic acid, $CH_2(OH).CH_2.COOH$, which is not found in the animal body and therefore has no physiological chemical interest. Indeed only α-oxypropionic acid or ethylidene lactic acid,  $\begin{matrix}CH_3\\ \dot{C}H(OH),\\ \dot{C}OOH\end{matrix}$ of which there are three physical isomeres, is of importance. These three ethylidene lactic acids are the ordinary, optically inactive FERMENTATION LACTIC ACID, the dextrorotatory PARALACTIC or SARCOLACTIC ACID, and the LEVOLACTIC ACID obtained by Schardinger by the fermentation of cane-sugar by means of a special bacillus. This levolactic acid, which has also been detected by Blachstein in the culture of Gaffky's typhoid bacillus in a solution of sugar and peptone, and which is formed by various vibriones, need not be described here.[1]

The *fermentation lactic acid*, which is formed from lactose by allowing milk to sour and by the acid fermentation of other carbohydrates, is considered to exist in small quantities in the muscles (Heintz), in the gray matter of the brain (Gscheidlen), and in diabetic urine. The occurrence of fermentation lactic acid in the brain and other organs has recently been disputed by Moriya.[2] During digestion this acid is also found in the contents of the stomach and intestine, and as alkali lactate in the chyle. The *paralactic acid* is, at all events, the true acid of meat extracts, and this alone has been found with certainty in dead muscle. The lactic acid which is found in the brain, spleen, lymphatic glands, thymus, thyroid gland, blood, bile, pathological transudates, osteomalacious bones, in perspiration in puerperal fever, in the urine after fatiguing marches, in acute yellow atrophy of the liver, in poisoning by phosphorus, and especially after extirpation of the liver seems always to be paralactic acid.

The origin of paralactic acid in the animal organism has been sought by several investigators, who took for basis the researches of Gaglio, Minkowski, and Araki, in a decomposition of protein in the tissues. Gaglio claims a lactic-acid formation by passing blood through the kidneys and lungs. He also found 0.3–0.5 p. m. lactic acid in the blood of a dog after protein food, and only 0.17–0.21 p. m. after fasting for forty-eight hours. According to Minkowski the quantity of lactic acid eliminated by the urine in animals with extirpated livers is increased with protein food, while the administration of carbohydrates has no effect. Araki has also shown that if we produce a scarcity of oxygen in animals (dogs, rabbits, and hens) by poisoning with carbon monoxide, by the inhalation of air deficient in oxygen, or by any other means, a considerable elimination of lactic acid (besides dextrose and also often albumin) takes place through the urine, an observation which has been confirmed by SAITO and KATSUYAMA.[1] As a scarcity of oxygen, according to the ordinary statements, produces an increase of the protein catabolism in the body, the increased elimination of lactic acid in these cases must be due in part to an increased protein destruction and in part to a diminished oxidation.

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[1] See Schardinger, Monatshefte f. Chem., **11**; Blachstein, Arch. des sciences biol. de St. Pétersbourg, **1**, 199; Kuprianow, Arch. f. Hygiene, **19**, and Gosio, *ibid.*, **21**.

[2] Heintz, Annal. d. Chem. u. Pharm., **157**, and Gscheidlen, Pflüger's Arch., **8**, 171; Moriya, Zeitschrift f. physiol. Chem., **43**.

ARAKI has not drawn such a conclusion from his experiments, but he considers the abundant formation of lactic acid to be due to a cleavage of the sugar formed from the glycogen. He found that in all cases where lactic acid and sugar appeared in the urine the quantity of glycogen in the liver and muscles was always diminished. He also calls attention to the fact that dextrolactic acid may be formed from glycogen, as directly observed by EKUNINA,[2] and also to the numerous observations on the formation of lactic acid and the consumption of glycogen in muscular activity. Without denying the possibility of a formation of lactic acid from protein, he states that with lack of oxygen we have to deal with an incomplete combustion of the lactic acid derived by a cleavage of the sugar. HOPPE-SEYLER[3] also positively defends the view as to the formation of lactic acid from carbohydrates. He was of the view that lactic acid is produced from the carbohydrates by the cleavage of the sugar only with lack of oxygen, while with sufficient oxygen the sugar is burned into carbon dioxide and water. The formation of lactic acid in the absence of free oxygen and in the presence of glycogen or dextrose is, according to HOPPE-SEYLER, very probably a function of all living protoplasm. In the anaerobic metabolism of the animal cells, according to the recent investigations on alcoholic fermentation in the tissues (see Chapters I and VIII), carbon dioxide and alcohol are formed from the sugar, with lactic acid as an intermediary step; but even if this view be correct and when the cells, as STOKLASA[4] and his collaborators have shown, contain a lactic-acid-forming enzyme, it is not known what kind of lactic acid is here produced. According to MORISHIMA an increase in the lactic acid in the liver occurs after death, probably from the liver glycogen, but this acid is chiefly fermentation lactic acid. ASHER and JACKSON[5] experimented by transfusing blood (with and without the addition of sugar) through the lower extremities of dogs, and neither in these experiments nor in those where the larger organs (liver and abdominal viscera) were excluded from the circulation could they detect any increase of lactic acid due to the sugar.

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[1] Gaglio, Arch. f. (Anat. u.) Physiol., 1886; Minkowski. Arch. exp. Path. u. Pharm., **21** and **31**; Araki, Zeitschr. f. physiol. Chem., **15**, **16**, **17**, and **19**; Saito and Katsuyama, *ibid.*, **32**.

[2] Journ. f. prakt. Chem. (N. F.), **21**.

[3] Virchow's Festschrift, also Ber. d. deutsch. chem. Gesellsch., **25**, Referatb., **685**.

[4] Simácek, Centralbl. f. Physiol., **17**; Stoklasa, Jelinek, and Cerny, *ibid.*, **16**.

[5] Morishima, Arch. f. exp. Path. u. Pharm., **43**; Asher and Jackson, Zeitschr. f. Biologie, **41**.

Although these last-mentioned investigations do not show any formation of lactic acid from carbohydrates, still, on the other hand, we have recent investigations that make such an origin for lactic acid very probable. Thus EMBDEN[1] has found, on percolating blood through a surviving liver rich in glycogen, that lactic acid was formed, and also that this acid was produced in abundance when blood rich in sugar was transfused through a glycogen-free liver, while, on the contrary, blood poor in sugar led to only a very inconsiderable formation of lactic acid. The investigations of A. R. MANDEL and LUSK[2] also indicate a formation of lactic acid from carbohydrates in the animal body. They have shown that in dogs, after phosphorus poisoning, an abundance of lactic acid occurs in the blood and urine, and that this disappears from these fluids on bringing about a phlorhizin diabetes in the animal. Phosphorus intoxication caused no lactic-acid formation in a phlorhizin-diabetic dog. Although it is difficult to give a satisfactory explanation of the results of these experiments, still it seems probable that by elimination of the sugar in phlorhizin diabetes a mother-substance of the lactic acid is lost.

The carbohydrates, as well as the proteins, it seems, must be considered as the material from which the lactic acid is formed in the body. In a previous chapter (VIII) we mentioned the formation of lactic acid in the animal body by a deamination of alanine, and this gives us an indication of a lactic acid formation from protein. Phosphocarnic acid is considered by SIEGFRIED as another source of sarcolactic acid.

The lactic acids are amorphous. They have the appearance of colorless or faintly yellowish, acid-reacting syrups which mix in all pro portions with water, alcohol, or ether. The salts are soluble in water, and most of them also in alcohol. The two acids are differentiated from each other by their different optical properties—paralactic acid being dextrogyrate, while fermentation lactic acid is optically inactive—also by their different solubilities and the different amounts of water of crystallization of the calcium and zinc salts. The zinc salt of fermentation lactic acid dissolves in 58–63 parts of water at 14–15° C., and contains 18.18 per cent water of crystallization, corresponding to the formula $Zn(C_3H_5O_3)_2+3H_2O$. The zinc salt of paralactic acid dissolves in 17.5 parts of water at the above temperature and contains ordinarily 12.9 per cent water, corresponding to the formula $Zn(C_3H_5O_3)_2+2H_2O$. The calcium salt of fermentation lactic acid dissolves in 9.5 parts water and contains 29.22 per cent (=5 molecules) water of crystallization, while calcium paralactate dissolves in 12.4 parts water and contains 24.83 or 26.21 per cent (=4 or 4½ molecules) water of crystallization. Both calcium salts crystallize, not unlike tyrosine, in spears or tufts of very fine microscopic needles. **Hoppe-Seyler** and **Araki**, who have closely studied the optical properties of the lactic acids and lactates, consider the lithium salt as best suited for the preparation and quantitative estimation of the lactic acids. The lithium salt contains 7.29 per cent Li. For further information as to the salts and specific rotation of the lactic acids see **Hoppe-Seyler-Thierfelder's** Handbuch, 7. Aufl., 1903.[1]

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[1] Centralbl. f. Physiol., 18, 832.

[2] Amer. Journ. of Physiol., **16**.

Lactic acids may be detected in organs and tissues in the following manner: After complete extraction with water, the protein is removed by coagulation at boiling temperature and the addition of a small quantity of sulphuric acid. The liquid is then exactly neutralized while boiling with caustic baryta, and then evaporated to a syrup after filtration. The residue is precipitated with absolute alcohol, and the precipitate completely extracted with alcohol. The alcohol is entirely distilled from the united alcoholic extracts, and the neutral residue is shaken with ether to remove the fat. The residue is dissolved in water and phosphoric acid added, and the solution repeatedly shaken with fresh quantities of ether, which dissolves the lactic acid. The ether is now distilled from the united ethereal extracts, the residue dissolved in water, and this solution carefully warmed on the water-bath to remove the last traces of ether and volatile acids. A solution of zinc lactate is prepared from this filtered solution by boiling with zinc carbonate, and this is evaporated until crystallization commences, and then is allowed to stand over sulphuric acid. An analysis of the salts is necessary in careful work. According to **Heffter**,[2] in muscles not having undergone rigor mortis the lactic acid can be extracted more easily by alcohol than by water.

Fat is never absent in the muscles. Some fat is always found in the intermuscular connective tissue; but the muscle-fibres themselves also contain fat. The quantity of fat in the real muscle substance is always small, usually amounting to about 10 p. m. or somewhat more. A considerable quantity of fat in the muscle-fibres is found only in fatty degeneration. A part of the muscle-fat can be readily extracted, while another part can be extracted only with the greatest difficulty. This latter part, it is claimed, exists finely divided in the contractile substance itself and is richer in free fatty acids, standing, according to **Zuntz** and **Bogdanow**,[3] in close relationship to the activity of the muscles because it is consumed during work. *Lecithin* is a regular constituent of the muscles, and it is quite possible that the fat which is difficult of extraction and which is rich in fatty acids depends in part on a decomposition of the lecithin. The amount of lecithin is not considerable. In normal dog-heart, as free from fat as possible, Rubow[1] found that the lecithin amounted to 7.5–8.5 per cent of the dry substance; for the striated muscle the amount of lecithin was rather constant, namely, 5.08 per cent. The ether extract of the heart of the dog contained 60–70 per cent lecithin.

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[1] See also E. Jungfleisch, Compt. rend., **139**, **140**, and **142**.

[2] Arch. f. exp. Path. u. Pharm., **38**.

[3] Arch. f. (Anat. u) Physiol., 1897.

The Mineral Bodies of the Muscles. The ash remaining after burning the muscle, which amounts to about 10–15 p. m., calculated on the moist muscle, is acid in reaction. The largest constituent of the ash is potassium, whose occurrence, according to Macallum, is restricted to the dark diagonal bundles, and phosphoric acid. Next in amount we have sodium and magnesium, and lastly calcium, chlorine, and iron oxide. Sulphates exist only as traces in the muscles, but are formed by the burning of the proteins of the muscles, and therefore occur in abundant quantities in the ash. The muscles contain such a large quantity of potassium and phosphoric acid that potassium phosphate seems to be unquestionably the predominating salt. Chlorine is found in such insignificant quantities that it is perhaps derived from a contamination with blood or lymph. The quantity of magnesium is, as a rule, considerably greater than that of calcium. Iron occurs only in very small amounts. Schmey[2] found variations between 0.0129 p. m. (rabbits) and 0.0793 p. m. (human), calculated on the fresh muscle substance. The heart-muscle was comparatively richer in iron, 0.06–0.109 p. m.

The importance of the various mineral bodies for the function of the muscles has been studied by several experimenters (Loeb, Lingle, Howell, Overton, Langendorff and Hueck, and others[3]). Further proof as to the previously discussed ion action of the electrolytes and the antagonism of various ions has been given by many very interesting investigations. These researches also indicate that each of the ions Na, Ca, and K plays a certain part in the maintenance of the excitability, in the contraction and in the fatigue of the muscle (heart); still these investigations have not led to concordant results, so that we are not yet clear on the action of these ions. Nevertheless it seems to be established that the combined action of various ions is a necessity for the normal function of the muscles. It has also been shown that it is possible to maintain the muscle (the heart) in regular activity for a long time by means of a transfusion of liquid saturated with oxygen and which contained about 7 p. m. NaCl, besides small amounts of $CaCl_2$ (0.2 p. m.), KCl (0.1 p. m.), and $NaHCO_3$ (0.1 p. m.).

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[1] Arch. f. exp. Path. u. Pharm., **52**.

[2] Macallum, Journ. of Physiol., **32**; Schmey, Zeitschr. f. physiol. Chem., **39**.

[3] Loeb, Amer. Journ. of Physiol., **3**, and Pflüger's Arch., **80**, **91**; Lingle, Amer. Journ. of Physiol., **4** (also references to literature); Overton, Pflüger's Arch., **92** and **105**; Langendorff and Hueck, *ibid.*, **96**.

The *gases* of the muscles consist of large quantities of carbon dioxide, besides traces of nitrogen.

In regard to the permeability of the muscles for various bodies there are the complete investigations of OVERTON.[1] The different sheaths of the muscles, the sarcolemma and perimysium internum, offer no very great resistance to the diffusion of the most soluble crystalloid compounds, while the muscle-fibres, on the contrary (exclusive of the sarcolemma), are almost if not entirely impervious to most inorganic compounds and to many organic compounds. The muscle-fibres themselves are actually semipermeable structures which are permeable for water but not for the molecules or ions of sodium chloride and of potassium phosphate. The muscle-fibres, as well as the various sheaths, are impermeable to colloids.

The behavior of the numerous bodies investigated cannot be discussed in this work. The general rule is as follows: All compounds which, besides having a marked solubility in water, are readily soluble in ethyl ether, in the higher alcohols, in olive-oil and in similar organic solvents, or are not much less soluble in the last-mentioned solvents than in water, pass through the living muscle-fibres with great ease. The greater the difference between the solubility of a compound in water and in the other solvents mentioned, the slower does the passage into the muscle-fibres take place. The permeability changes essentially on the death of the muscle.

The living muscle-fibres are readily permeable to oxygen, carbon dioxide, and ammonia, while the hexoses and disaccharides do not readily pass into them. It is very remarkable that a great portion of those compounds which take part in the normal metabolism of plants and animals belong to those bodies to which the muscle-fibres (and also other cells) are entirely or at least nearly impermeable. On the contrary, derivatives can be prepared from these bodies which pass into the cells very readily, and OVERTON finds that it is not impossible that the organism in part makes use of a similar artifice in order to regulate the concentration of the nutritive bodies within the protoplasm.

Rigor Mortis of the Muscles. If the influence of the circulating oxygenated blood is removed from the muscles, as after the death of the animal or by ligature of the aorta or the muscle-arteries (STENSON'S test), *rigor mortis* sooner or later takes place. The ordinary rigor appearing under these circumstances is called the spontaneous or the fermentative rigor, because it seems to depend in part on the action of an enzyme. A muscle may also become stiff for other reasons. The muscles may become momentarily stiff by warming, in the case of frogs to 40°, in mammalia to 48–50°, and in birds to 53° C. The heat-rigor depends upon the coagulation of certain proteins, and its occurrence at lower temperatures in cold-blooded as compared with warm-blooded animals is due, according to v. FÜRTH, to the fact that in the first a soluble myogen fibrin occurs preformed in the muscle which coagulates at 30–40° C., while in the warm-blooded animals the coagulating substance is musculin (myosin of v. FÜRTH) which coagulates at a higher temperature. Distilled water may also produce a rigor in the muscles (water-rigor). Acids, even very weak ones, such as carbon dioxide, may quickly produce a rigor (acid-rigor), or hasten its appearance. A number of chemically different substances, such as chloroform, ether, alcohol, ethereal oils, caffeine, and many alkaloids, produce a similar effect. The rigor which is produced by means of acids or other agents which, like alcohol, coagulate proteins must be considered as produced by entirely different processes from those causing spontaneous rigor.

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[1] Pflüger's Arch., 92. See also Höber, *ibid.*, 106, and Hamburger, Osmotischer Druck und Ionenlehre, Bd. 3.

When the muscle passes into rigor mortis it becomes shorter and thicker, harder and non-transparent, and less ductile. The acid part of the amphoteric reaction becomes stronger, which is explained by most investigators by the assumption of a formation of lactic acid. There is hardly any doubt that this increase in acidity may at least in part be due to a transformation of a part of the diphosphate into monophosphate by the lactic acid. The statements in regard to the presence or absence of free lactic acid in the rigor-mortis muscle are contradictory.[1] Besides the formation of acid, the chemical processes which take place in rigor of the muscles are the following: By the coagulation of the plasma a myosin-clot is produced which is the cause of the hardening and of the diminished transparency of the muscle; but this view must be changed on account of the researches of v. FÜRTH, which have shown that the clot consists of myogen fibrin and myosin fibrin. The appearance of this clot may be hastened by the simultaneous occurrence of lactic acid. Carbon dioxide is also formed, which does not seem to be a direct oxidation product, but a product of the cleavage processes. HERMANN[2] claims that carbon dioxide is produced in the removed muscle, even in the absence of oxygen, when it passes into rigor mortis. In connection with this view we must call attention to FOLIN's[3] observations that no protein coagulation took place in rigor under special conditions.

As many investigators admit of an increased formation of lactic acid on the appearance of rigor mortis, the question arises, from what constituents of the muscle is this acid derived? The most probable explanation is that the lactic acid is produced from the glycogen, as certain investigators, such as Nasse and Werther, have observed a decrease in the quantity of glycogen in rigor of the muscle. On the other side, Böhm[1] has observed cases in which no consumption of glycogen took place in rigor of the muscle, and he has also found that the quantity of lactic acid produced is not proportional to the quantity of glycogen. It is therefore possible that the consumption of glycogen and the formation of lactic acid in the muscles are two processes independent of each other, and, as above stated in regard to the formation of paralactic acid, the lactic acid of the muscle may be considered as a decomposition product of protein. The origin of the carbon dioxide is also not to be sought for in the decomposition of the glycogen or dextrose. Pflüger and Stintzing[2] have found that in the muscle a substance occurs which evolves large quantities of carbon dioxide on boiling with water, and it is probably this substance which is decomposed with the formation of carbon dioxide in tetanus as well as in rigor. In this connection we call attention to the fact that phosphocarnic acid yields lactic acid as well as carbon dioxide as cleavage products.

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[1] It is impossible to enter into the details of the disputed statements as to the reaction of the muscles, etc. We shall only refer to the works of Röhmann, Pflüger's Arch., **50** and **55**, and Heffter, Arch. f. exp. Path. u. Pharm., **31** and **38**. These works contain also the researches of the older investigators more or less completely.

[2] Untersuchungen über den Stoffwechsel der Muskeln, etc., Berlin, 1867.

[3] Amer. Journ. of Physiol., **9**.

After the muscles have been rigid for some time they relax again and become softer. This is in part produced by the strong acid dissolving the myosin-clot and in part by autolytic processes (Vogel[3]).

Metabolism in the Inactive and Active Muscles. It is admitted by a number of prominent investigators, Pflüger and Colasanti, Zuntz and Röhrig,[4] and others, that the metabolism in the muscles is regulated by the nervous system. When at rest, when there is no mechanical exertion, there exists a condition which Zuntz and Röhrig have designated "*chemical tonus*." This tonus seems to be a reflex tonus, for it may be reduced by discontinuing the connection between the muscles and the central organ of the nervous system by cutting through the spinal cord or the muscle-nerves. The possibility of reducing the chemical tonus of the muscles in various ways offers an important means of deciding the extent and kind of chemical processes going on in the muscles when at rest. In comparative chemical investigation of the processes in the active and the inactive muscles several methods of procedure have been adopted. The same active and inactive muscles have been compared after removal, also the arterial and venous muscle-blood in rest and activity, and lastly the total exchange of material, the receipts and expenditures of the organism, have been investigated under these two conditions.

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[1] Nasse, Beitr. z. Physiol. der kontrakt. Substanz, Pflüger's Arch., **2**; Werther, *ibid.*, **46**; Böhm, *ibid.*, **23** and **46**.

[2] Pflüger's Arch., **18**.

[3] R. Vogel, Unters. über Muskelsaft, Deutsch. Arch. f. klin. Med., 1902.

[4] See the works of Pflüger and his pupils in Pflüger's Arch., **4**, **12**, **14**, **16**, and **18**; Röhrig, *ibid.*, **4**. See also Zuntz, *ibid.*, **12**. In regard to the metabolism after curare poisoning, see also Frank and Voit, Zeitschr. f. Biologie, **42**, and Frank and Gebhard, *ibid.*, **43**.

By investigations according to these several methods it has been found that the active muscle takes up oxygen from the blood and returns to it carbon dioxide, and also that the quantity of oxygen taken up is greater than the oxygen contained in the carbon dioxide eliminated at the same time. The muscle, therefore, holds in some form of combination a part of the oxygen taken up while at rest. During activity the exchange of material in the muscle, and therewith the exchange of gas, is increased. The animal organism takes up much more oxygen in activity than when at rest, and eliminates also considerably more carbon dioxide. The quantity of oxygen which leaves the body as carbon dioxide during activity is much larger than the quantity of oxygen taken up at the same time; and the venous muscle-blood is poorer in oxygen and richer in carbon dioxide during activity than during rest. The exchange of gases in the muscles during activity is the reverse of that at rest, for the active muscle gives up a quantity of carbon dioxide which does not correspond to the quantity of oxygen taken up, but is considerably greater. It follows from this that in muscular activity not only does oxidation take place, but also splitting processes occur. This results also from the fact that removed blood-free muscles when placed in an atmosphere devoid of oxygen can labor for some time and also yield carbon dioxide (HERMANN[1]).

During muscular inactivity, in the ordinary sense, a consumption of glycogen takes place. This is inferred from the observations of several investigators that the quantity of glycogen is increased and its corresponding consumption reduced in those muscles whose chemical tonus is reduced either by cutting through the nerve or for other reasons (BERNARD, CHANDELON, VAY,[2] and others). In activity this consumption of glycogen is increased, and it has been positively proved by the researches of several investigators (NASSE, WEISS, KÜLZ, MARCUSE, MANCHÉ, MORAT and DUFOUR[3]) that the quantity of glycogen in the muscles in activity decreases quickly and freely. As shown by the researches of CHAUVEAU and KAUFMANN, QUINQUAUD, MORAT and DUFOUR, CAVAZZANI, and especially those of SEEGEN,[4] the sugar is removed from the blood and consumed during activity. According to SEEGEN a very abundant formation of sugar takes place in the liver, and correspondingly the blood of the hepatic vein is much richer in sugar than that in the portal vein; and this sugar of the blood is, according to him, the source of heat formation and mechanical activity. It is nevertheless true that important objections have been presented against a few of these investigations, and a sugar formation, according to SEEGEN'S idea, has been denied by several investigators, and recently by ZUNTZ and MOSSE; but still there can exist hardly any doubt that sugar is consumed in muscular activity. A direct proof for this has recently been given by JOH. MÜLLER.[1] In experiments on surviving cats' hearts which were percolated with a salt solution containing sugar, he could detect an undoubted consumption of sugar which was quite considerable.

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[1] l. c. In regard to gas exchange in removed muscles, see also J. Tissot, Arch. de Physiol. (5), **6** and **7**, and Compt. rend., **120**.

[2] Chandelon, Pflüger's Arch., **13**; Vay, Arch. f. exp. Path. u. Pharm., **34**, which also contains the pertinent literature.

[3] Nasse, Pflüger's Arch., **2**; Weiss, Wien. Sitzungsber., **64**; Külz, in Ludwig's Festschrift, Marburg, 1890; Marcuse, Pflüger's Arch., **39**; Manché, Zeitschr. f. Biologie, **25**; Morat and Dufour, Arch. de Physiol. (5), **4**.

[4] Chauveau and Kaufmann, Compt. rend., **103**, **104**, and **105**; Quinquaud, Maly's Jahresber., **16**; Morat and Dufour, l. c.; Cavazzani, Centralbl. f. Physiol., **8**; Seegen, "Die Zuckerbildung im Thierkörper," Berlin, 1890, Centralbl. f. Physiol., **8**, **9**, and **10**; Arch. f. (Anat. u.) Physiol., 1895 and 1896; Pflüger's Arch., **50**.

The amphoteric reaction of the inactive muscles is changed during activity to an acid reaction (DU BOIS-REYMOND and others), and the acid reaction increases to a certain point with the work. The quickly contracting pale muscles produce, according to GLEISS,[2] more acid during activity than the more slowly contracting red muscles. The acid reaction appearing during activity was formerly considered to be due to the formation of lactic acid, a view which has been contradicted by ASTASCHEWSKY, PFLÜGER, and WARREN, who found less lactic acid in the tetanized muscle than when at rest. MONARI also found a decrease in the quantity of lactic acid during activity, and according to HEFFTER the quantity of lactic acid in the muscle is diminished in tetanus produced by poison. Contrary to these investigations, MARCUSE and WERTHER have been able to prove the formation of lactic acid during activity; still the statements are very contradictory. Other observations indicate a formation of lactic acid during activity. Thus SPIRO found an increase in the quantity of lactic acid in the blood during work. COLASANTI and MOSCATELLI found small quantities of lactic acid in human urine after strenuous marches, and WERTHER observed an abundance of lactic acid in the urine of frogs after tetanization. According to HOPPE-SEYLER, on the contrary, in agreement with his view in regard to the formation of lactic acid, lactic acid is not produced regularly during work, but only when insufficient oxygen is supplied. ZILLESEN [3] has also found that on artificially cutting off the oxygen from the muscles during life more lactic acid was formed than under normal conditions.

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[1] Mosse, Pflüger's Arch., 63; Zuntz, Centralbl. f. Physiol., 10, and Arch. f. (Anat. u.) Physiol., 1896, 538. See also Schenck, Pflüger's Arch., 61 and 65; Müller, Zeitschr. f. allgem. Physiol., 3.

[2] Pflüger's Arch., 41.

[3] Astaschewsky, Zeitschr. f. physiol. Chem., 4; Warren, Pflüger's Arch., 24; Monari, Maly's Jahresber., 19; Heffter, Arch. f. exp. Path. u. Pharm., 31; Marcuse, l. c.; Werther, Pflüger's Arch., 46; Spiro, Zeitschr. f. physiol. Chem., 1; Colasanti and Moscatelli, Maly's Jahresber., 17, 212; Hoppe-Seyler, l. c., and Zeitschr. f. physiol. Chem., 19; Zillesen, *ibid.*, 15.

It is evident that the experiments with the muscles *in situ*—in other words, with muscles through which blood is passing—cannot yield any conclusion to the above question, as the lactic acid formed during work may perhaps be removed by the blood. The following objections can be made against those experiments in which lactic acid has been found after moderate work in the blood or the urine, as also especially against the experiments with removed active muscles, namely, that in these cases the supply of oxygen to the muscles was not sufficient, and that the lactic acid formed thereby is not, in accordance with the views of HOPPE-SEYLER, a perfectly normal process. The question as to the formation of lactic acid in the active muscle under perfect physiological conditions is still an open one, although several observations make it seem to be very probable.

According to SIEGFRIED the amount of phosphocarnic acid is diminished during activity. MACLEOD claims that this is true only for intense muscular activity, while with ordinary work the organic phosphorus not present as nucleons is diminished and the quantity of phosphates is increased. This stands in accord with WEYL and ZEITLER's[1] observations that the active muscle contains more phosphoric acid than the inactive muscle. As in the dead muscle, so in the active muscle, the somewhat stronger acid reaction is in part due to a greater quantity of monophosphate.

The amount of proteins in the removed muscles is, according to the older investigators, decreased by work. The correctness of this statement is, however, disputed by other investigators. The older statements in regard to the nitrogenous extractive bodies of the muscle in rest and in activity are likewise uncertain. According to the recent researches of MONARI[2] the total quantity of creatine and creatinine is increased by work, and indeed the amount of creatinine is especially augmented by an excess of muscular activity. The creatinine is formed essentially from the creatine. In excessive activity MONARI also found xanthocreatinine in the muscle, and the quantity was one-tenth that of the creatinine. The purine bases are, according to BURIAN,[3] increased during work, due to a greater formation (see above, page 454). It seems to have been positively shown that the active muscle contains a smaller quantity of bodies soluble in water and a larger quantity of bodies soluble in alcohol than the resting muscle. (HELMHOLTZ[4]).

Attempts have been made to solve the question relative to the behavior of the nitrogenized constituents of the muscle at rest and during activity by determining the total quantity of nitrogen eliminated under these different conditions of the body. While formerly it was held with LIEBIG that the elimination of nitrogen by the urine was increased by muscular work, the researches of several experimenters, especially those of VOIT on dogs and PETTENKOFER and VOIT on men, have led to quite different results. They have shown, as has also lately been confirmed by other investigators, especially I. MUNK and HIRSCHFELD,[1] that during work no increase or only a very insignificant increase in the elimination of nitrogen takes place.

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[1] Siegfried, Zeitschr. f. physiol. Chem., **21**; Macleod, *ibid.*, **28**; Weyl and Zeitler, *ibid.*, **6**.

[2] Maly's Jahresber., **19**, 296.

[3] Zeitschr. f. physiol. Chem., **43**.

[4] Arch. f. (Anat. u.) Physiol., 1845.

We should not omit to mention the fact that a series of experiments has been made showing a significant increase in the metabolism of proteins during or after work. There are for example the observations of FLINT and of PAVY on a pedestrian, v. WOLFF, v. FUNKE, KREUZHAGE, and KELLNER on a horse, and DUNLOP and his collaborators on working human beings, and of KRUMMACHER, PFLÜGER, ZUNTZ and his pupils,[2] and others. The researches on the elimination of sulphur during rest and activity also belong to this category. The elimination of nitrogen and sulphur runs parallel with the metabolism of proteins in resting and active persons, and the quantity of sulphur excreted by the urine is therefore also a measure of the protein catabolism. The older researches of ENGELMANN, FLINT, and PAVY, as well as the more recent ones of BECK and BENEDICT,[3] and DUNLOP and his collaborators, show an increased elimination of sulphur during or after work, and this indicates an increased protein metabolism because of muscular activity.

That an increased destruction of protein is not necessarily produced by work follows from the observations of CASPARI, BORNSTEIN, KAUP, WAIT, A. LOEWY, ATWATER and BENEDICT,[4] that a retention of nitrogen and a deposition of protein occur during work. The contradictory observations on the protein destruction during and caused by work are not directly in opposition to each other, because the extent of protein metabolism is dependent upon many conditions, such as the quantity and composition of the food, the condition of the adipose tissue of the body, the action of the work upon the respiratory mechanism, etc., all of which have an influence on the results of the experiments.

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[1] Voit, Untersuchungen über den Einfluss des Kochsalzes, des Kaffees und der Muskelbewegungen auf den Stoffwechsel (München, 1860), and Zeitschr. f. Biologie, **2**; J. Munk, Arch. f. (Anat. u.) Physiol., 1890 and 1896; Hirschfeld, Virchow's Arch., **121**.

[2] Flint, Journ. of Anat. and Physiol., **11** and **12**; Pavy, The Lancet, 1876 and 1877; v. Wolff, v. Funke, Kellner, cited from Voit, Hermann's Handb., **6**, 197; Dunlop, Nool-Paton, Stockman, and Maccadam, Journ. of Physiol., **22**; Krummacher, Zeitschr. f. Biologie, **33**; Pflüger, Pflüger's Arch., **50**; Zuntz, Arch. f. (Anat. u.) Physiol., 1894.

[3] Engelmann, Arch. f. (Anat. u.) Physiol., 1871; Beck and Benedict, Pflüger's Arch., **54**, and also foot-note 2.

[4] Caspari, Pflüger's Arch., **83**; Bornstein, *ibid.*; Kaup, Zeitschr. f. Biologie, **43**; Wait, U. S. Depart. Agricult. Bulletin **89** (1901); Atwater and Benedict, *ibid.*, Bull. **69** (1899); Loewy, Arch. f. (Anat. u.) Physiol., 1901.

Recently STEYRER[1] has found that the muscle juice of a continuously tetanized muscle was somewhat poorer in musculin and correspondingly richer in myogen than the juice from a similar non-tetanized muscle. We cannot draw any conclusions from this experiment, but it seems to show that the proteins are not consumed in work.

The older investigations on the amount of fat in muscles removed after activity and after rest have not led to any definite results. According to the recent investigations of ZUNTZ and BOGDANOW,[2] the fat belonging to the muscle-fibres and which is difficultly extracted takes part in work. Besides these there are several researches by VOIT, PETTENKOFER and VOIT, J. FRENTZEL,[3] and others which make an increased destruction of fat during work probable.

If the results of the investigations thus far made of the chemical processes going on in the active and inactive muscle were collected together, we would find the following characteristics for the active muscle. The active muscle takes up more oxygen and gives off more carbon dioxide than the inactive muscle; still the elimination of carbon dioxide is increased considerably more than the absorption of oxygen. The respiratory quotient, $\frac{CO_2}{O}$, is found to be regularly raised during work; yet this rise, which will be explained in detail in a following chapter on metabolism, can hardly be conditioned on the kind of processes going on in the muscle during activity with a sufficient supply of oxygen. In work a consumption of carbohydrates, glycogen, and sugar takes place. The acid reaction of the muscle becomes greater with work. In regard to the extent of a re-formation of lactic acid opinion is divided. An increased consumption of fat has occasionally been observed. The quantity of organic phosphorus decreases, and an increase in the nitrogenous extractives of the creatinine group seems also to occur. Protein metabolism has been found increased in certain series of experiments and not in others; but an increased elimination of nitrogen as a direct consequence of muscular exertion has thus far not been positively proved.

In close connection with the above-mentioned facts there is the question as to the material basis of muscular activity so far as it has its origin in chemical processes. In the past the generally accepted opinion was that of LIEBIG, that the source of muscular action consisted of a catabolism of the protein bodies; to-day another generally accepted view prevails. FICK and

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[1] Hofmeister's Beiträge, 4.

[2] Arch. f. (Anat. u.) Physiol., 1897.

[3] Pflüger's Arch., 68.

WISLICENUS[1] climbed the Faulhorn and calculated the amount of mechanical force expended in the attempt. With this they compared the mechanical equivalent transformed in the same time from the proteins, calculated from the nitrogen eliminated with the urine, and found that the work really performed was not by any means compensated by the consumption of protein. It was therefore proved by this that proteins alone cannot be the source of muscular activity, and that this depends in great measure on the metabolism of non-nitrogenous substances. Many other observations have led to the same result, especially the experiments of VOIT, of PETTENKOFER and VOIT, and of other investigators, whose observations show that while the elimination of nitrogen remains unchanged, the elimination of carbon dioxide during work is very considerably increased. It is also generally considered as positively proved that muscular work is produced, at least in greatest part, by the catabolism of non-nitrogenous substances. Nevertheless there is no warrant for the statement that muscular activity is produced entirely at the cost of the non-nitrogenous substances, and that the protein bodies are without importance as a source of energy.

The investigations of PFLÜGER[2] are of great interest in this connection. He fed a bulldog for more than seven months with meat which alone did not contain sufficient fat and carbohydrates even for the production of heart activity, and then let him work very hard for periods of 14, 35, and 41 days. The positive result obtained by these series of experiments was that "complete muscular activity may be effected to the greatest extent in the absence of fat and carbohydrates," and the ability of proteins to serve as a source of muscular energy cannot be denied.

The nitrogenous as well as the non-nitrogenous nutriments may serve as a source of energy; but the views are divided in regard to the relative value of these. PFLÜGER claims that no muscular work takes place without a decomposition of protein, and the living cell-substance prefers always the protein and rejects the fat and sugar, contenting itself with these only when proteins are absent. Other investigators, on the contrary, believe that the muscles first draw on the supply of non-nitrogenous nutriments, and according to SEEGEN, CHAUVEAU, and LAULANIÉ[3] the sugar is indeed the only direct source of muscular force. The last-mentioned investigator holds that the fat is not directly utilized for work, but only after a previous conversion into sugar. ZUNTZ and his collaborators have made strong objections to the correctness of such a view. If, according to ZUNTZ, the fat must be first transformed into sugar before it can serve as the source of muscular work, a definite expenditure of force must require about 30 per cent more energy with fatty food than it does with carbohydrates; but this is not the case. The investigations of ZUNTZ, (together with) LOEB, HEINEMANN, FRENTZEL and REACH show that all foodstuffs have nearly the same power of serving as the material for the work of the muscles. The extensive metabolism investigations of ATWATER and BENEDICT[1] have also led to similar results as to the fats being a source of muscular energy. The law of the substitution of the foodstuffs, according to their combustion equivalents, is also true for muscular work, and fat correspondingly acts with its full amount of energy without previously being transformed into sugar. The question which of the foodstuffs the muscle prefers is dependent upon the relative quantities of the same at the disposal of the muscle. A direct substitution of the body material by the bodies supplied as food does not take place in the muscular activity in the ordinary nutritive condition. According to JOHANSSON and KORAEN[2] the CO_2 excretion produced by certain work is not influenced by the supply of foodstuffs (protein or sugar).

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[1] Vierteljahrsschr. d. Zürich. naturf. Gesellsch., **10**, cited from Centralbl. f. d. med. Wiss., 1866, 309.

[2] Pflüger's Arch., **50**.

[3] See Seegen, foot-note 4, page 468. The works of Chauveau and his collaborators are found in Compt. rend., **121**, **122**, and **123**; Laulanié, Arch. de Physiol. (5), **8**.

SIEGFRIED considers, as above stated, the phosphocarnic acid as a source of energy. According to his and KRÜGER'S[3] researches, phosphocarnic acid, which yields on cleavage, among other bodies, carbon dioxide, occurs in part preformed in the muscle, and in part as a hypothetical aldehyde compound of the same—a compound which forms phosphocarnic acid on oxidation. SIEGFRIED therefore makes the suggestion that in the resting muscle, which requires more oxygen than exists in the carbon dioxide eliminated, this reducing aldehyde substance is gradually oxidized to phosphocarnic acid, which is used in the activity of the muscle with the splitting off of carbon dioxide.

Quantitative Composition of the Muscle. A large number of analyses have been made of the flesh of various animals for purely practical purposes, in order to determine the nutritive value of different varieties of meat; but there are no exact scientific analyses with sufficient regard to the quantity of different protein bodies and the remaining muscle constituents, that is, these analyses are incomplete or of little value.

To give the reader some idea of the variable composition of muscle-substance the following summary is presented, chiefly obtained from K. B. HOFMANN'S[4] book, although it does not correspond to the present demands. The figures are parts per 1000.

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[1] Loeb, Arch. f. (Anat. u.) Physiol., 1894; Heinemann, Pflüger's Arch., **83**; Frentzel and Reach, *ibid.*; Atwater and Benedict, U. S. Dept. of Agric., Bull. 136, and Ergebnisse der Physiologie, **3**.

[2] Skand. Arch. f. Physiol., **13**.

[3] Zeitschr. f. physiol. Chem., **22**.

[4] Lehrbuch d. Zoochemie (Wien, **1876**), 104.

	Muscles of Mammals.	Muscles of Birds.	Muscles of Cold-blooded Animals.
Solids	217–255	225–282	200
Water	745–783	717–773	800
Organic bodies	208–245	217–263	180–190
Inorganic bodies	9–10	10–19	10–20
Myosin	35–106	29.8–111	29.7–87
Stroma substance (DANILEWSKY)	78–161	88.0–184	70.0–121
Creatine	2	3.4	2.3
Xanthine bodies	1.3–1.7	0.7–1.3	—
Inosinic acid (barium salt)	0.1	0.1–0.3	—
Protic acid	—	—	7.0
Taurine	0.7 (horse)	—	1.1
Inosite	0.03	—	—
Glycogen	4–37	—	3–5
Lactic acid	0.4–0.7	—	—
Phosphoric acid	3.4–4.8		
Potash	3.0–4.0		
Soda	0.3		
Lime	0.2		
Magnesia	0.4		
Sodium chloride	0.04–0.1		
Iron oxide	0.04–0.1		

In this table, which has little value because of the variation in the composition of the muscles, no results are given as to the estimates of fat. Owing to the variable quantity of fat in meat and the incompleteness of the older methods of estimation it is hardly possible to quote a positive average for this substance. After most careful efforts to remove the fat from the muscles without chemical means, it has been found that a variable quantity of intermuscular fat, which does not really belong to the muscular tissue, always remains. The smallest quantity of fat in the muscles from lean oxen is 6.1 p. m. according to GROUVEN, and 7.6 p. m. according to PETERSEN. This last observer also found regularly a smaller quantity of fat, 7.6–8.6 p. m., in the fore quarters of oxen, and a greater amount, 30.1–34.6 p. m., in the hind quarters of the animal, but this could not be substantiated by STEIL.[1] A small quantity of fat has also been found in the muscles of wild animals. B. KÖNIG and FARWICK found 10.7 p. m. fat in the muscles of the extremities of the hare, and 14.3 p. m. in the muscles of the partridge. The muscles of pigs and fattened animals are, when all the adherent fat is removed, very rich in fat, amounting to 40–90 p. m. The muscles of certain fishes also contain a large quantity of fat. According to ALMÉN, in the flesh of the salmon, the mackerel, and the eel there are contained respectively 100, 164, and 329 p. m. fat.[2]

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[1] See Steil, Pflüger's Arch., 61.

[2] In regard to the literature and complete statements on the composition of flesh of various animals, see König, Chemie der menschlichen Nahrungs- und Genussmittel, 5. Aufl.

The quantity of *water* in the muscle is liable to considerable variation. The quantity of fat has a special influence on the quantity of water, and one finds, as a rule, that the flesh which is deficient in water is correspondingly rich in fat. The quantity of water does not depend alone upon the amount of fat, but upon many other circumstances, among which must be mentioned the age of the animal. In young animals, the organs in general, and therefore also the muscles, are poorer in solids and richer in water. In man the quantity of water decreases until mature age, but increases again towards old age. Work and rest also influence the quantity of water, for the active muscle contains more water than the inactive. The uninterruptedly active heart should therefore be the muscle richest in water. That the quantity of water may vary independently of the amount of fat is strikingly shown by comparing the muscles of different species of animals. In cold-blooded animals the muscles generally have a greater quantity of water, in birds a lower. The comparison of the flesh of cattle and fish shows very strikingly the different amounts of water (independent of the quantity of fat) in the flesh of different animals. According to the analysis of ALMÉN,[1] the muscles of lean oxen contain 15 p. m. fat and 767 p. m. water; the flesh of the pike contains only 1.5 p. m. fat and 839 p. m. water.

For certain purposes, as, for example, in experiments on metabolism, it is important to know the elementary composition of flesh. In regard to the quantity of nitrogen we generally accept VOIT's figure, namely, 3.4 per cent, as an average for fresh lean meat. According to NOWAK and HUPPERT[2] this quantity may vary about 0.6 per cent, and in more exact investigations it is therefore necessary to specially determine the nitrogen. Complete elementary analyses of flesh have been made with great care by ARGUTINSKY. The average for ox-flesh dried *in vacuo* and free from fat and with the glycogen deducted was as follows: C 49.6; H 6.9; N 15.3; O+S 23.0; and ash 5.2 per cent. KÖHLER found as an average for water and fat-free beef C 49.86; H 6.78; N 15.68; O+S 22.3 per cent, which are very similar results. This investigator has also made similar analyses of the flesh of various animals and has determined the calorific value of the ash- and fat-free dried meat substance. This value was, per gram of substance, 5.599–5.677 Cal. The relationship of the carbon to nitrogen, which ARGUTINSKY calls the "*flesh quotient*," is on an average 3.54:1. From KÖHLER's analyses the average for beef is 3.15:1 and for horse-flesh 3.38:1. According to SALKOWSKI, of the total nitrogen of beef 77.4 per cent was insoluble proteins, 10.08 per cent soluble proteins, and 12.52 per cent other soluble bodies. FRENTZEL and SCHREUER[1] find that about 7.74 per cent of the total nitrogen belongs to the nitrogenous extractives.

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[1] Nova Act. Reg. Soc. Scient. Upsal., Vol. extr. ord., 1877; also Maly's Jahresber., 7.

[2] Voit, Zeitschr. f. Biologie, 1; Huppert, *ibid.*, 7; Nowak, Wien. Sitzungsber., 64, Abt. 2.

There exist complete investigations by KATZ[2] as to the quantity of mineral constituents of the muscles from man and animals. The variation in the different elements is considerable. Pork is much richer in sodium as compared with potassium than other kinds of meat. The quantity of magnesium is greater, and often considerably greater, than calcium in all kinds of flesh investigated, with the exception of the haddock, the eel, and the pike. Beef is very poor in calcium. Potassium and phosphoric acid are the most abundant mineral constituents of all flesh.

Non-striated Muscles.

The smooth muscles have a neutral or alkaline reaction (DU BOIS-REYMOND) when at rest. During activity they are acid, which is inferred from the observations of BERNSTEIN, who found that the almost continually contracting sphincter muscle of the Anodonta is acid during life. The smooth muscles may also, according to HEIDENHAIN and KÜHNE, pass into rigor mortis and thereby become acid. A spontaneous but slowly coagulating plasma has also been observed in several cases.

In regard to the proteins of the smooth muscles we have the older statements of HEIDENHAIN and HELLWIG;[3] but they were first carefully studied according to newer methods by MUNK and VELICHI.[4] These experimenters have prepared a neutral plasma from the gizzard of geese, according to v. FÜRTH's method. This plasma coagulated spontaneously at the temperature of the room, although slowly. It contained a *globulin*, precipitated by dialysis, which coagulated at 55–60° C. and also showed certain similarities with KÜHNE's myosin. A spontaneously coagulating *albumin*, which differed from myogen (v. FÜRTH) by coagulating at 45–50° C., and which passes by spontaneous coagulation into the coagulated modification without a soluble intermediate product, exists in still greater quantities in this plasma. Alkali albuminates do not occur, but a *nucleoproteid* is found, which exists in about five times the quantity as compared with striated muscles. Nucleon is, according to PANELLA,[5] a normal constituent of smooth muscles and occurs in larger amounts than in striated muscles.

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[1] Argutinsky, Pflüger's Arch., 55; Köhler, Zeitschr. f. physiol. Chem., **31**; Salkowski, Centralbl. f. d. med. Wissensch., 1894; Frentzel and Schreuer, Arch. f. (Anat u.) Physiol., 1902.

[2] Pflüger's Arch., **63**. See also Schmey, Zeitschr. f. physiol. Chem., **39**.

[3] Du Bois-Reymond in Nasse, Hermann's Handb., **1**, 339; Bernstein, *ibid.*; Heidenhain, *ibid.*, 340, with Hellwig, *ibid.*, 339; Kühne, Lehrbuch, 331.

[4] Munk and Velichi, Centralbl. f. Physiol., **12**.

[5] Maly's Jahresber., **34**.

Recent investigations of **Bottazzi** and **Cappelli**, **Vincent** and **Lewis Vincent**, and v. **Fürth**,[1] some on the muscles of warm-blooded and some on those of lower animals, have led to somewhat contradictory results, but they substantiate, as a whole, the observations of **Munk** and **Velichi**. Besides the nucleoproteids the smooth muscles contain two bodies corresponding in coagulation temperature to musculin and myosinogen (myogen, v. **Fürth**), but they are not identical therewith. *Hæmoglobin* occurs in the smooth muscles of certain animals, but is absent in others. In the smooth muscles (in certain varieties of animals) *creatine*, *creatinine*, *taurine*, *inosite*, *glycogen*, and *lactic acid* have been found. The mineral constituents show the remarkable fact that the sodium compounds exceed the potassium compounds.

Henze found abundance of taurine in the muscles of octopods, 5 p. m., but no creatine, which, according to **Frémy** and **Valenciennes**,[2] occurs in the muscles of cephalopods. He also found no glycogen and no paralactic acid, but, on the contrary, small amounts of fermentation lactic acid. The muscles of octopods are richer in mineral bodies than the muscles of vertebrates, and are nearly twice as rich in sulphur as these.

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[1] Bottazzi, Centralbl. f. Physiol., **15**; Vincent and Lewis, Journ. of Physiol., **26**; Vincent, Zeitschr. f. physiol. Chem., **34**; v. Fürth, *ibid.*, **31**.

[2] Henze, *ibid.*, **43**; Frémy and Valenciennes, cited from Kühne's Lehrbuch, p. 333.

CHAPTER XII.

BRAIN AND NERVES.

On account of the difficulty in making a mechanical separation and isolation of the different tissue-elements of the central nervous organ and the nerves, we must resort to a few microchemical reactions, chiefly to qualitative and quantitative investigations of the different parts of the brain, in order to study the varied chemical composition of the cells and the nerve-axes. This study is accompanied with the greatest difficulty; and although our knowledge of the chemical composition of the brain and nerves has been somewhat extended by the investigations of modern times, still it must be admitted that this subject is as yet one of the most obscure and complicated in physiological chemistry.

Proteins of different kinds have been shown to be chemical constituents of the brain and nerves, and these are representatives of the same chief groups as occur in the protoplasm. In the brain there occur some proteins which are insoluble in water and neutral salt solutions, and which resemble the stroma substances of the muscles and cells, while other proteins are soluble in water and neutral salt solutions. Among the latter we find chiefly *nucleoproteids* and *globulins*. The nucleoproteid found by HALLIBURTON and also by LEVENE[1] in the gray substance contains 0.5 per cent phosphorus and coagulates at 55–60°. LEVENE obtained adenine and guanine but no hypoxanthine as cleavage products. According to HALLIBURTON there are two globulins, namely, the neuroglobulin α, which coagulates at 47° or at 50–53° in the case of birds, and the neuroglobulin β, whose coagulation temperature is 70–75°, but which varies somewhat in different animals. In the frog still another protein body occurs, which coagulates at a still lower temperature, about 40°. It must be remarked that the coagulation temperature of α-globulin corresponds with the temperature of the first heat contraction of the nerves of different classes of animals (HALLIBURTON).

Just as there are lecithin-albumins, compounds of proteid with lecithin, so according to ULPIANI and LELLI[1] there exists an analogous compound in the brain which is a combination between a protagon-like substance and a pseudonuclein.

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[1] Halliburton, On the Chemical Physiology of the Animal Cell, King's College, London, Physiological Laboratory, Collected Papers No. 1, 1893, and Ergebnisse der Physiologie, 4; Levene, Arch. of Neurology and Psychopathology, 2 (1899).

There does not seem to be any doubt that the proteins belong chiefly to the gray substance of the brain and to the axis-cylinders. The same remark also applies to the *nuclein*, which v. JACKSCH[2] found in large quantities in the gray substance. *Neurokeratin*, which was first detected by KÜHNE, and which partly forms the *neuroglia*, and as a double sheath envelops the outside of the nerve-medulla under SCHWANN'S sheath and the inner axis-cylinders, occurs in the nerves, but chiefly, or according to KOCH entirely, in the white substance (KÜHNE and CHITTENDEN, BAUMSTARK[3]).

The phosphorized substance *protagon* must be considered as one of the chief constituents, perhaps the only constituent (BAUMSTARK), of the white substance. This last-mentioned substance, if we keep for the present to the most carefully studied protagon—because there are perhaps several different protagons—yields as decomposition products lecithin, fatty acids, and a nitrogenous substance, *cerebrin*. It is difficult to state whether this last body also exists preformed in the brain. At least an allied substance, *cerebron*, occurs preformed in the brain. That *lecithin* also is pre-existent in the brain and nerves can hardly be doubted. The investigations thus far made have not shown decisively whether it is more abundant in the gray or the white substance; according to KOCH it is much more abundant in the white substance. *Fatty acids* and *neutral fats* may be prepared from the brain and nerves; but as these may be readily derived from a decomposition of lecithin and protagon, which exist in the fatty tissue between the nerve-axes, it is difficult to decide what part the fatty acids and neutral fats play as constituents of the real nerve-substance. *Cholesterin* seems chiefly, and according to KOCH perhaps entirely, to occur in the white substance. Besides these substances the nerve-tissue, especially the white substance, contains doubtless a number of other constituents not well known, and among which are several containing phosphorus. THUDICHUM,[4] who has made thorough investigations of the brain and has described a great number of brain constituents, has given the name *phosphatides* to all substances of the brain containing the phosphoric-acid radical. Those phosphatides which contain only one phosphoric-acid radical are called monophosphatides, those with two such radicals diphosphatides. The monophosphatides can contain one, two, or more nitrogen atoms in their molecule, while there are also nitrogen-free monophosphatides. Irrespective of the relation between phosphorus and nitrogen, certain phosphatides differ from the lecithins by not yielding any glycerophosphoric acid. These investigations of THUDICHUM are without doubt of great importance, but as they have not been repeated we cannot enter into a discussion of the bodies described by him.

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[1] Cited from Chem. Centralbl., 1902, 2, 292.

[2] Pflüger's Arch., 13.

[3] Koch, Amer. Journ. of Physiol., 11; Kühne and Chittenden, Zeitschr. f. Biologie **26**; Baumstark, Zeitschr. f. physiol. Chem., 9.

[4] Thudichum, Die chemische Konstitution des Gehirns des Menschen und der Tiere, Tübingen, 1901.

By allowing water to act on the contents of the medulla, round or oblong double-contoured drops or fibres, not unlike double-contoured nerves, are formed. These remarkable formations, which can also be seen in the medulla of the dead nerve, have been called "*myeline forms*," and they were formerly considered as produced from a special body, "myeline." Myeline forms may, however, be obtained from other bodies, such as impure protagon, lecithin, fat, and impure cholesterin, and they depend upon a decomposition of the constituents of the medulla. According to GAD and HEYMANS[1] myeline is lecithin in a free condition or in loose chemical combination.

The *extractive bodies* seem to be almost the same as in the muscles. One finds *creatine*, which may, however, be absent (BAUMSTARK), *xanthine bodies*, *inosite*, *choline*, *paralactic acid* (MORIYA), *phosphocarnic acid*, *uric acid*, *jecorin* (according to BALDI,[2] in the human brain), and the diamine *neuridine*, $C_5H_{14}N_2$, discovered by BRIEGER[3] and which is most interesting because of its appearance in the putrefaction of animal tissues or in cultures of the typhoid bacillus. Under pathological conditions *leucine* and *urea* have been found in the brain. Urea is also a physiological constituent of the brain of cartilaginous fishes.

Of the above-mentioned constituents of the nerve-substance protagon and the cerebrins or cerebrosides must be specially described.

Protagon. This body, which was discovered by LIEBREICH, is a nitrogenized and phosphorized substance whose elementary composition, according to GAMGEE and BLANKENHORN, is C 66.39, H 10.69, N 2.39, and P 1.068 per cent. BAUMSTARK and RUPPEL obtained the same figures, while LIEBREICH found an average of 2.80 per cent N and 1.23 per cent P. KOSSEL and FREYTAG, who obtained still higher figures for the nitrogen, namely, 3.25 per cent, and somewhat lower figures for the phosphorus, 0.97 per cent, found some sulphur, an average of 0.51 per cent, regularly in the protagon. RUPPEL also found some sulphur, but in such small quantity that he considered it as a contamination. CRAMER has prepared by an essentially different method a protagon which contained sulphur but had in other respects the same composition as that analyzed by GAMGEE and BLANKENHORN. POSNER and GIES obtained, in a very extensive investigation, fractions which had variable compositions. These last investigators, as well as THUDICHUM, LESEM, WÖRNER and THIERFELDER, and KOCH,[1] are therefore of the opinion that protagon does not exist as a chemical individual, but is a mixture, essentially of cerebrins, lecithin, and cephalin. The somewhat variable elementary composition also indicates the fact that the protagon as ordinarily obtained is not a homogeneous substance. On the contrary, the assumption that protagon is only a mixture of cerebrins and lecithin-like bodies is, according to HAMMARSTEN, very improbable. That a mixture of amorphous or very difficultly crystallizable substances produces so readily such a beautifully crystalline substance as protagon, which can be recrystallized as often as one wishes, contradicts the ordinary chemical experience. What seems more probable is that the so-called protagon is a crystalline substance which can be purified from other substances, perhaps its own decomposition products, with the very greatest difficulty.

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[1] Arch. f. (Anat. u.) Physiol., 1890.

[2] Baldi, Arch. f. (Anat. u.) Physiol., 1887, Suppl.; Moriya, Zeitschr. f. physiol. Chem., 43.

[3] Brieger, Ueber Ptomaine, Berlin, 1885 and 1886.

As we are not decided whether protagon is only a mixture or is a body contaminated with other substances, it is difficult to decide as to how far the so-called decomposition products exist as preformed constituents of the mixture or whether they are true decomposition products. On boiling with baryta-water protagon yields the decomposition products of lecithin, namely, fatty acids, glycerophosphoric acid, and choline. KOSSEL and FREYTAG found indeed three cerebrosides, namely, CEREBRIN, KERASIN (homocerebrin), and ENCEPHALIN.

On boiling with dilute mineral acids protagon yields among other substances a reducing carbohydrate. On oxidation with nitric acid protagon yields higher fatty acids.

Protagon appears, when dry, as a loose white powder. It dissolves in alcohol of 85 vols. per cent at 45° C., but separates on cooling as a snow-white, flaky precipitate, consisting of balls or groups of fine crystalline needles. It decomposes on heating even below 100° C. It is hardly soluble in cold alcohol or ether, but dissolves, at least when freshly precipitated, in ether on warming. It swells in little water and partly decomposes. With more water it swells to a gelatinous or pasty mass, which with much water yields an opalescent liquid. On fusing with saltpetre and soda, alkali phosphates are obtained.

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[1] Gamgee and Blankenhorn, Zeitschr. f. physiol. Chem., **3**; Baumstark, l. c.; Ruppel, Zeitschr. f. Biologie, **31**; Liebreich, Annal. d. Chem. u. Pharm., **134**; Kossel and Freytag, Zeitschr. f. physiol. Chem., **17**; Wörner and Thierfelder, *ibid.*, **30**; Lesem and Gies, Amer. Journ. of Physiol., 8; Thudichum, l. c.; Cramer, Journ. of Physiol., **31**; Posner and Gies, Journ. of Biolog. Chem., **1**.

Protagon is prepared in the following way: An ox-brain as fresh as possible, with the blood and membranes carefully removed, is ground fine and then extracted for several hours with alcohol of 85 vols. per cent at 45° C., filtered at the same temperature, and the residue extracted with warm alcohol until the filtrate does not yield a precipitate at 0° C. The several alcoholic extracts are cooled to 0° C. and the precipitates united and completely extracted with cold ether, which dissolves the cholesterin and lecithin-like bodies. The residue is now strongly pressed between filter-paper and allowed to dry over sulphuric acid or phosphoric anhydride. It is now pulverized, digested with alcohol at 45° C., filtered, and slowly cooled to 0° C. The crystals which separate may be purified when necessary by recrystallization.

The same steps are taken when one wishes to detect the presence of protagon.

On decomposing protagon (or the protagons) by the gentle action of alkalies we obtain as cleavage products, as above stated, one or more bodies which THUDICHUM has embraced under the name *cerebrosides.* The cerebrosides are nitrogenous substances free from phosphorus, which yield a reducing variety of sugar (galactose) on boiling with dilute mineral acids. On fusing with potash or by oxidation with nitric acid they yield higher fatty acids—palmitic or stearic acids. The cerebrosides isolated from the brain are cerebrin, kerasin, encephalin, and cerebron, but it must be remarked that there is no doubt but that sometimes the same body of varying purity has received different names. The bodies isolated by KOSSEL and FREYTAG from pus, and called pyosin and pyogenin, also belong to the cerebrosides.

Cerebrin. Under this name W. MÜLLER[1] first described a nitrogenous substance, free from phosphorus, which he obtained by extracting with boiling alcohol a brain-mass which had been previously boiled with baryta-water. Following a method essentially the same, but differing somewhat, GEOGHEGAN[2] prepared from the brain a cerebrin with the same properties as MÜLLER'S, but containing less nitrogen. According to PARCUS[3] the cerebrin isolated by GEOGHEGAN, as well as by MÜLLER, consists of a mixture of three bodies, "cerebrin," "homocerebrin," and "encephalin." KOSSEL and FREYTAG isolated two cerebrosides from protagon which were identical with the cerebrin and homocerebrin of PARCUS. According to these investigators, the two bodies phrenosin and kerasin, as described by THUDICHUM, seem to be identical with cerebrin and homocerebrin.

Cerebrin, according to PARCUS, has the following composition: C 69.08, H 11.47, N 2.13, O 17.32 per cent, which corresponds with the analyses made by KOSSEL and FREYTAG. No formula has been given to this body.

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[1] Annal. d. Chem. u. Pharm., **105.**

[2] Zeitschr. f. physiol. Chem., **3.**

[3] Parcus, Ueber einige neue Gehirnstoffe, Inaug.-Diss. Leipzig, 1881.

In the dry state it forms a pure white, odorless, and tasteless powder. On heating it melts, decomposes gradually, smells like burnt fat, and burns with a luminous flame. It is insoluble in water, dilute alkalies, or baryta-water; also in cold alcohol and in cold or hot ether. On the contrary, it is soluble in boiling alcohol and separates as a flaky precipitate on cooling, and this is found to consist of a mass of balls or grains on microscopical examination. Cerebrin forms a compound with baryta, which is insoluble in water and is decomposed by the action of carbon dioxide. Cerebrin dissolves in concentrated sulphuric acid, and on warming the solution it becomes blood-red. The variety of sugar split off on boiling with mineral acids—the so-called brain-sugar—is, according to THIERFELDER,[1] galactose.

Kerasin (according to THUDICHUM), or *homocerebrin* (according to PARCUS), has the following composition: C 70.06, H 11.60, N 2.23, and O 16.11 per cent. **Encephalin** has the composition C 68.40, H 11.60, N 3.09, and O 16.91 per cent. Both bodies remain in the mother-liquor after the impure cerebrin has precipitated from the warm alcohol. These bodies have the tendency of separating as gelatinous masses. Kerasin is similar to cerebrin, but dissolves more easily in warm alcohol and also in warm ether. It may be obtained as extremely fine needles. Encephalin is, according to PARCUS, a transformation product of cerebrin. In the perfectly pure state it crystallizes in small lamellæ. It swells into a pasty mass in warm water. Like cerebrin and kerasin, it yields a reducing substance (probably galactose) on boiling with dilute acid.

The cerebrins are generally prepared according to MÜLLER's method. The brain is first stirred with baryta-water until it appears like thin milk, and then it is boiled. The insoluble parts are removed, pressed, and repeatedly boiled with alcohol, which is filtered while boiling hot. The impure cerebrin which separates on cooling is freed from cholesterin and fat by means of ether and then purified by repeated solution in warm alcohol. According to PARCUS this repeated solution in alcohol is continued until no gelatinous separation of homocerebrin or encephalin takes place.

According to GEOGHEGAN's method the brain is first extracted with cold alcohol and ether and then boiled with alcohol. The precipitate which separates on the cooling of the alcoholic filtrate is treated with ether and then boiled with baryta-water. The insoluble residue is purified by repeated solution in boiling alcohol.

The cerebrin may also be obtained from other organs by employing the above methods. The quantitative estimation, when such is desired, may be performed in the same way.

KOSSEL and FREYTAG prepare cerebrin from protagon by saponifying it in methyl alcohol solution with a hot solution of caustic baryta in methyl alcohol. The precipitate is filtered off and decomposed in water by carbon dioxide and the cerebrin or cerebroside extracted from the insoluble residue with hot alcohol.

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[1] Zeitschr. f. physiol. Chem., **14**.

Whether the above-described cerebrins are chemical individuals or mixtures, i.e., impure substances, is still undecided. The purest cerebrin or cerebroside thus far investigated is undoubtedly THIERFELDER's cerebron, and there is hardly any doubt also that MÜLLER's cerebrin consisted essentially of cerebron.

Cerebron. This cerebrin, isolated by THIERFELDER and WÖRNER and then especially studied by THIERFELDER, was first isolated by GAMGEE and called *pseudocerebrin* by him. THUDICHUM's [1] *phrenosin* seems to be impure cerebron. Cerebron can be prepared directly from the brain without saponification with baryta, by treatment with alcohol containing benzene or chloroform at a temperature of 50°, and hence it is considered as existing preformed in the brain. According to THIERFELDER cerebron has the formula $C_{48}H_{93}NO_9$; it melts at 212°, dissolves in warm alcohol, and separates out on cooling. From proper solvents (acetone containing chloroform) it may be separated as small needles or plates. If cerebron is suspended in 85 per cent alcohol at a temperature of 50° C. it balls together in amorphous masses, and from these needle- and leaf-shaped crystals gradually form. Cerebron also yields galactose, and it can be split by acids, best in methyl alcohol containing sulphuric acid, into galactose, a base called *sphingosin* by THUDICHUM, and *cerebronic acid* (THUDICHUM's *neurostearic acid*). The cleavage takes place, according to THIERFELDER, as follows: $C_{48}H_{93}NO_9+2H_2O=C_{25}H_{50}O_3$ (cerebronic acid) $+C_{17}H_{35}NO_2$ (sphingosin) $+C_6H_{12}O_6$ (galactose). The cerebronic acid consists of snow-white crystals which are soluble in alcohol and in ether. They melt at 99–100° and give a crystalline methyl ester which melts at 65°. Sphingosin is a base which does not form marked crystals and which is insoluble in water and ether, but gives a sulphate which is soluble in chloroform and in warm alcohol. According to THIERFELDER and KITAGAWA, sphingosin is not a unit substance.[2]

Cephalin is a phosphatide whose formula, based upon the investigations of THUDICHUM and KOCH [3] is probably $C_{42}H_{82}NPO_{13}$. The views of these two investigators as to the constitution of this body, which is difficult to purify, differ very considerably. According to THUDICHUM, on cleavage it yields neurine, glycerophosphoric acid, stearic acid, and a specific fatty acid, *cephalic acid.* According to KOCH it contains on the contrary, only one methyl group attached to nitrogen, and is therefore probably dioxystearyl-monomethyl lecithin. Cephalin is amorphous and swells up in water like lecithin. It is soluble in cold ether, glacial acetic acid, and chloroform, but is insoluble in acetone and in alcohol, either cold or warm. It is obtained from the brain after dehydration with acetone by extracting with ether and precipitating the concentrated ethereal extract with alcohol. The cephalin is perhaps identical with the myeline substance isolated by ZUELZER[1] from the brain.

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[1] Thierfelder and Wörner, Zeitschr. f. physiol. Chem., **30**; Thierfelder, *ibid.*, **43**, **44**, and **46**; Gamgee, Text-book of Physiol. Chem., London, 1880; Thudichum, l. c.

[2] Zeitschr. f. physiol. Chem., 48.

[3] Thudichum, l. c.; Koch, Zeitschr. f. physiol. Chem., **36**.

BETHE[2] has prepared the following decomposition products from the brain of the horse after treatment with $CuCl_2$ and alkali: *aminocerebrinic-acid glucoside*, $C_{44}H_{81}O_8N$, which on boiling with hydrochloric acid yields cerebrinic acid, aminocerebrinic-acid chloride, and a hexose (galactose ?); *phrenin*, perhaps identical with THUDICHUM's krinosin; *cerebrinic-phosphoric acid*, and a *stearic acid* differing somewhat from the ordinary one.

Neuridine, $C_5H_{14}N_2$, is a non-poisonous diamine discovered by BRIEGER, and which was obtained by him in the putrefaction of meat and gelatine, and from cultures of the typhoid bacillus. It also occurs under physiological conditions in the brain, and as traces in the yolk of the egg.

Neuridine dissolves in water and yields on boiling with alkalies a mixture of dimethylamine and trimethylamine. It dissolves with difficulty in amyl alcohol. It is insoluble in ether or absolute alcohol. In the free state, neuridine has a peculiar odor, suggesting semen. With hydrochloric acid it gives a compound crystallizing in long needles. With platinic chloride or gold chloride it gives crystallizable double compounds which are valuable in its preparation and detection.

The so-called CORPUSCULA AMYLACEA, which occur on the upper surface of the brain and in the pituitary gland, are colored more or less pure violet by iodine and more blue by sulphuric acid and iodine. They consist, perhaps, of the same substance as certain prostatic calculi, but they have not been closely investigated.

Quantitative Composition of the Brain. The quantity of water is greater in the gray than in the white substance, and greater in new-born or young individuals than in adults. The brain of the fœtus contains 879–926 p. m. water. According to the observations of WEISBACH[3] the quantity of water in the several parts of the brain (and in the medulla) varies at different ages. The following figures are in 1000 parts—*A* for men and *B* for women:

	20–30 Years.		30–50 Years.		50–70 Years.		70–94 Years.	
	A.	*B.*	*A.*	*B.*	*A.*	*B.*	*A.*	*B.*
White brain-substance..	695.6	682.9	683.1	703.1	701.9	689.6	726.1	722.0
Gray " .	833.6	826.2	836.1	830.6	838.0	838.4	847.8	839.5
Gyri.	784.7	792.0	795.9	772.9	796.1	796.9	802.3	801.7
Cerebellum.	788.3	794.9	778.7	789.0	787.9	784.5	803.4	797.9
Pons Varolii.	734.6	740.3	725.5	722.0	720.1	714.0	727.4	724.4
Medulla oblongata.	744.3	740.7	732.5	729.8	722.4	730.6	736.2	733.7

Quantitative analyses have also been made of the ox-brain by PETROWSKY,[4] and of the brain of a horse by BAUMSTARK. In the analysis of PETROWSKY the protagon has not been considered, and all organic phosphorized substances were calculated as lecithin. On these grounds these analyses are not of much value from a certain standpoint. In **Baumstark's** analyses the gray and the white substance could not be sufficiently separated, and these analyses, on this account, show partly an excess of white and partly an excess of gray substance; nearly one-half of the organic bodies, chiefly consisting of bodies soluble in ether, could not be exactly analyzed. Neither of these analyses gives sufficient explanation of the quantitative composition of the brain.

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[1] W. Koch, Zeitschr. f. physiol. Chem., **36**; Zuelzer, *ibid.*, **27**.

[2] Arch. f. exp. Path. u. Pharm., **48**.

[3] Cited from K. B. Hofmann's Lehrb. d. Zoochemie (Wien, 1876), 121.

[4] Pflüger's Arch., **7**.

The analyses made up to the present time give, as above stated, an unequal division of the organic constituents in the gray and white substance. In the analyses of **Petrowsky** the quantity of proteins and gelatine-forming substances in the gray matter was somewhat more than one-half, and in the white about one-quarter of the solid organic substances. The quantity of cholesterin in the white was about one-half, and in the gray substance about one-fifth of the solid bodies. A greater quantity of soluble salts and extractive bodies was found in the gray substance than in the white (**Baumstark**). The following analyses of **Baumstark** give the most important known constituents of the brain calculated in 1000 parts of the fresh, moist substance. *A* represents chiefly the white, and *B* chiefly the gray substance.

	A.	*B.*
Water	695.35	769.97
Solids	304.65	230.03
Protagon	25.11	10.80
Insoluble protein and connective tissue	50.02	60.79
Cholesterin, free	18.19	6.30
" combined	26.96	17.51
Nuclein	2.94	1.99
Neurokeratin	18.93	10.43
Mineral bodies	5.23	5.62

The remainder of the solids probably consists chiefly of lecithin and other phosphorized bodies. Of the total amount of phosphorus 15–20 p. m. belongs to the nuclein, 50–60 p. m. to the protagon, 150–160 p. m. to the ash, and 770 p. m. to the lecithin and the other phosphorized organic substances.

As shown by the above analysis **Baumstark** differentiated between free and combined cholesterin. He believed that a part of the cholesterin in the brain occurred in the combined state, perhaps as an ester; this view has been found to be incorrect by the recent investigations of **Bünz**. He obtained from the brain neither esters of cholesterin with higher fatty acids nor other compounds of cholesterin which split on saponification. **Tebb**[1] has also found only free cholesterin.

The analysis of the brain of an epileptic made by **Koch**[2] is of very great interest. We cannot enter into a discussion of his method of analysis.

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[1] Bünz, Zeitschr. f. physiol. Chem., **46**; Tebb, Journ. of Physiol., **34**.

[2] Amer. Journ. of Physiol., **11**; Koch and Woods, Journ. of Biol. Chem., **1**.

In order to make the figures found comprehensible, it is perhaps necessary to call attention to a few points. The two cerebrins, phrenosin and kerasin, were calculated from the quantity of galactose split off. The quantity of phosphatides, designated by Koch as lecithans, was determined from the quantity of methyl groups split off by hydriodic acid below 240° plus the quantity of true lecithin calculated from the quantity of methyl groups split off at about 300°. The difference between the quantity of lecithin and the total quantity of lecithans gave the amount of cephalin and myelin. The nature of the sulphurized substance is unknown. As the protagon, according to Koch, is a mixture of various substances, no results as to the quantity is given. The other figures require no explanation.

	Corpus Callosum	Cortex (prefrontal).
Water	67.97	84.13
Protein	3.20	5.00
Nucleoproteids	3.70	3.00
Neurokeratin	2.70 (Chittenden)	0.40 (Chittenden)
Extractives (water-soluble)	1.51	1.58
Lecithins	5.19	3.14
Cephalin and myelin	3.49	0.74
Phrenosin and kerasin	4.57	1.55
Cholesterin	4.86	0.70
Sulphurized substance	1.40	1.45
Mineral bodies	0.82	0.87

As the cerebrosides occur chiefly in the myelin sheath, Koch, starting from the amount in the investigated part of the brain, attempts to calculate the amount of the analyzed cortical substance in the white nerves, and on the basis of these calculations, he finds the following values for the pure gray substance, free from nerve-fibres, and compares them with the corpus callosum. The results are in 100 parts of the dry substance.

	Corpus Callosum.	Gray Substance (free from white substance).
Protein	10.00	21.70
Nucleoproteids	11.56	9.66
Neurokeratin	8.40	—
Extractives	4.75	5.92
Lecithins	16.22	7.67
Cephalin and myelin	10.91	—
Phrenosin and kerasin	14.29	—
Cholesterin	15.20	—
Sulphurized substance	4.37	5.43

According to Noll the white substance of the spinal marrow is somewhat richer in protagon than the brain, and in nerve degeneration the quantity of protagon diminishes. The method used by him would not allow of an exact determination of the protagon. Mott and Halliburton[1] have also shown that in degenerative diseases of the nervous system the quantity of substances containing phosphorus diminishes and that in these cases, especially in general paralysis, choline passes into the cerebrospinal fluid and the blood. In degenerated nerves, the quantity of water increases and the phosphorus decreases.

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[1] Noll, Zeitschr. f. physiol. Chem., **27**; Mott and Halliburton, Philos. Transact., Ser. B, **191** (1899) and **194** (1901).

The quantity of neurokeratin in the nerves and in the different parts of the brain has been carefully determined by KÜHNE and CHITTENDEN.[1] They found 3.16 p. m. in the plexus brachialis, 3.12 p. m. in the cortex of the cerebellum, 22.434 p. m. in the white substance of the cerebrum, 25.72–29.02 p. m. in the white substance of the corpus callosum, and 3.27 p. m. in the gray substance of the cortex of the cerebrum (when free as possible from white substance). The white is decidedly richer in neurokeratin than the peripheral nerves or the gray substance. According to GRIFFITHS,[2] *neurochitin* replaces neurokeratin in insects and crustacea, the quantity of the first being 10.6–12 p. m.

The quantity of mineral constituents in the brain amounts to 2.95–7.08 p. m. according to GEOGHEGAN. He found in 1000 parts of the fresh, moist brain 0.43–1.32 Cl; 0.956–2.016 PO_4; 0.244–0.796 CO_3; 0.102–0.220 SO_4; 0.01–0.098 $Fe_2(PO_4)_2$; 0.005–0.022 Ca; 0.016–0.072 Mg; 0.58–1.778 K; 0.450–1.114 Na. The gray substance yields an alkaline ash, the white an acid ash.

Appendix.

THE TISSUES AND FLUIDS OF THE EYE.

The retina contains in all 865–899.9 p. m. water, 57.1–84.5 p. m. protein bodies—myosin, albumin, and mucin (?), 9.5–28.9 p. m. lecithin, and 8.2–11.2 p. m. salts (HOPPE-SEYLER and CAHN [3]). The mineral bodies consist of 422 p. m. Na_2HPO_4 and 352 p. m. NaCl.

Those bodies which form the different segments of the rods and cones have not been closely studied, and the greatest interest is therefore connected with the coloring-matters of the retina.

Visual purple, also called *rhodopsin*, *erythropsin*, or VISUAL RED, is the pigment of the rods. BOLL [4] observed in 1876 that the layer of rods in the retina during life had a purplish-red color which was bleached by the action of light. KÜHNE [5] showed later that this red color might remain for a long time after the death of the animal if the eye was protected from daylight or investigated by a sodium light. Under these conditions it was also possible to isolate and closely study this substance.

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[1] Zeitschr. f. Biologie, 26.

[2] Compt. rend.. 115.

[3] Zeitschr. f. physiol. Chem., 5.

[4] Monatsschr. d. Kgl. Preuss. Akad., 12. Nov., 1876.

[5] The investigations of Kühne and his pupils, Ewald and Ayres, on the visual purple will be found in Untersuchungen aus dem physiol. Institut der Universität Heidelberg, 1 and 2, and in Zeitschr. f. Biologie, 32.

Visual red (Boll) or visual purple (Kühne) has become known mainly by the investigations of Kühne. The pigment occurs chiefly in the rods and only in their outer parts. In animals whose retina has no rods the visual purple is absent, and is also necessarily absent in the macula lutea. In a variety of bat (Rhinolophus hipposideros), in hens, pigeons and new-born rabbits, no visual purple has been found in the rods.

A solution of visual purple in water which contains 2–5 per cent crystallized bile, which is the best solvent for it, is purple-red in color, quite clear, and not fluorescent. On evaporating this solution *in vacuo* we obtain a residue similar to ammonium carminate which contains violet or black grains. If the above solution is dialyzed with water, the bile diffuses and the visual purple separates as a violet mass. Under all circumstances, even when still in the retina, the visual purple is quickly bleached by direct sunlight, and with diffused light with a rapidity corresponding to the intensity of the light. It passes from red and orange to yellow. Red light bleaches the visual purple slowly; the ultra-red light does not bleach it at all. A solution of visual purple shows no special absorption-bands, but only a general absorption which extends from the red side, beginning at *D* and extending to the *G* line. The strongest absorption is found at *E*.

Koettgen and Abelsdorf[1] have shown that there are, in accordance with Kühne's views, two varieties of visual purple, the one occurring in mammals, birds, and amphibians, and the other, which is more violet-red, in fishes. The first has its maximum absorption in the green and the other in the yellowish green.

Visual purple when heated to 52–53° C. is destroyed after several hours, and almost instantly when heated to 76° C. It is also destroyed by alkalies, acids, alcohol, ether, and chloroform. On the contrary, it resists the action of ammonia or alum solution.

As the visual purple is easily destroyed by light, it must therefore also be regenerated during life. Kühne has also found that the retina of the eye of the frog becomes bleached when exposed for a long time to strong sunlight, and that its color gradually returns when the animal is placed in the dark. This regeneration of the visual purple is a function of the living cells in the layer of the pigment-epithelium of the retina. This may be inferred from the fact that a detached piece of the retina which has been bleached by light may have its visual purple restored if it is carefully laid on the choroid having layers of the pigment-epithelium attached. The regeneration has, it seems, nothing to do with the dark pigment, the melanin or fuscin, in the epithelium-cells. A partial regeneration seems, according to Kühne, to be possible in the retina which has been completely removed. On account of this property of the visual purple of being bleached by light during life we may, as KÜHNE has shown, under special conditions and by observing special precautions, obtain after death, by the action of intense light or more continuous light, the picture of bright objects, such as windows and the like—so-called optograms.

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[1] Centralbl. f. Physiol., **9**; also Maly's Jahresber., **25**, 351.

The physiological importance of visual purple is unknown. It follows that the visual purple is not essential to sight, since it is absent in certain animals and also in the cones.

Visual purple must always be prepared exclusively in a sodium light. It is extracted from the net membrane by means of a watery solution of crystallized bile. The filtered solution is evaporated *in vacuo* or dialyzed until the visual purple is separated. To prepare a visual-purple solution perfectly free from hæmoglobin the solution of visual purple in cholates is precipitated by saturating with magnesium sulphate, washing the precipitate with a saturated solution of magnesium sulphate, and then dissolving in water by the aid of the cholates simultaneously precipitated.[1]

The Pigments of the Cones. In the inner segments of the cones of birds, reptiles, and fishes a small fat-globule of varying color is found. KÜHNE[2] has isolated from this fat a green, a yellow, and a red pigment called respectively *chlorophan*, *xanthophan*, and *rhodophan*.

The dark pigment of the epithelium-cells of the net membrane, which was formerly called *melanin*, but has since been named *fuscin* by KÜHNE and MAYS,[3] contains iron, dissolves in concentrated caustic alkalies or concentrated sulphuric acid on warming, but, like the melanins in general, has been little studied. The pigment occurring in the pigment-cells of the choroid will be discussed with the melanins in Chapter XVI.

The **vitreous humor** is often considered as a variety of gelatinous tissue. The membrane consists, according to C. MÖRNER, of a gelatine-forming substance. The fluid contains a little proteid and a mucoid, *hyalomucoid*, which was first shown by MÖRNER, and which is precipitated by acetic acid. This contains 12.27 per cent N and 1.19 per cent S. Among the extractives we find a little *urea*—according to PICARD 5 p. m., according to RÄHLMANN 0.64 p. m. PAUTZ[4] found besides some urea *paralactic acid*, and, in confirmation of the statements of CHABBAS, JESNER, and KUHN, also *glucose* in the vitreous humor of oxen. The reaction of the vitreous humor is alkaline, and the quantity of solids amounts to about 9–11 p. m. The quantity of mineral bodies is about 6–9 p. m. and the proteins 0.7 p. m. In regard to the aqueous humor see page 264.

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[1] Kühne, Zeitschr. f. Biologie, 32.

[2] Kühne, Die nichtbeständigen Farben der Netzhaut, Untersuch. aus dem physiol. Institut Heidelberg, 1, 341.

[3] Kühne, ibid., 2, 324.

[4] Mörner, Zeitschr. f. physiol. Chem., 18; Picard, cited from Gamgee, Physiol. Chem., 1, 454; Rählmann, Maly's Jahresber., 6; Pautz, Zeitschr. f. Biologie, 31. A complete review of the literature will also be found here.

The Crystalline Lens. That substance which forms the capsule of the lens has been investigated by C. MÖRNER. It belongs, according to him, to a special group of proteins, called *membranins*. The membranin bodies are insoluble at the ordinary temperature in water, salt solutions, dilute acids, and alkalies, and, like the mucins, yield a reducing substance on boiling with dilute mineral acids. They contain lead-blackening sulphur. The membranins are colored a very beautiful red by MILLON's reagent, but give no characteristic reaction with concentrated hydrochloric acid or ADAMKIEWICZ's reagent. They are dissolved with great difficulty by pepsin-hydrochloric acid or trypsin solution, but are soluble in dilute acids and alkalies in the warmth. Membranin of the capsule of the lens contains 14.10 per cent N and 0.83 per cent S, and is a little less soluble than that from DESCEMET's membrane.

The chief mass of the solids of the crystalline lens consists of proteins, whose nature has been investigated by C. MÖRNER.[1] Some of these proteins dissolve in dilute salt solution, while others remain insoluble in this solvent.

The Insoluble Protein. The lens-fibres consist of a protein substance which is insoluble in water and in salt solution and to which MÖRNER has given the name *albumoid*. It dissolves readily in very dilute acids or alkalies. Its solution in caustic potash of 0.1 per cent is very similar to an alkali-albuminate solution, but coagulates at about 50° C. on nearly complete neutralization and the addition of 8 per cent NaCl. Albumoid has the following composition: C 53.12, H 6.8, N 16.62, and S 0.79 per cent. The lens-fibres themselves contain 16.61 per cent N and 0.77 per cent S. The inner parts of the lens are considerably richer in albumoid than the outer. The quantity of albumoid in the entire lens amounts on an average to about 48 per cent of the total weight of the proteins of the lens.

The Soluble Protein consists, exclusive of a very small quantity of *albumin*, of two globulins, α- and *β-crystallin*. These two globulins differ from each other in this manner: α-crystallin contains 16.68 per cent N and 0.56 per cent S; β-crystallin, on the contrary, 17.04 per cent N and 1.27 per cent S. The first coagulates at about 72° C. and the other at 63° C. Besides this, β-crystallin is precipitated from a salt-free solution with greater difficulty and less completely by acetic acid or carbon dioxide. These globulins are not precipitated by an excess of NaCl at either the ordinary temperature or 30° C. Magnesium or sodium sulphate in substance precipitates both globulins, on the contrary, at 30° C. These two globulins are not equally divided in the mass of the lens. The quantity of α-crystallin diminishes in the lens from without inwards; β-crystallin, on the contrary, from within outwards.

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[1] Zeitschr. f. physiol. Chem., 18. This contains also the pertinent literature.

A. **Béchamp** distinguishes the two following protein bodies in the watery extract of the crystalline lens: *phacozymase*, which coagulates at 55° C., contains a diastatic enzyme, and has a specific rotatory power of $(\alpha)j = -41^{\circ}$, and the *crystalbumin*, with a specific rotatory power of $(\alpha)j = -80.3^{\circ}$. From the residue of the lens, which was insoluble in water, **Béchamp** extracted, by means of hydrochloric acid, a protein body having a specific rotatory power of $(\alpha)j = -80.2^{\circ}$, which he called *crystalfibrin*.

The lens does not seem to contain any protein bodies which coagulate spontaneously like fibrinogen. That cloudiness which appears after death depends, according to Kühne, upon the unequal changing of the concentration of the contents of the lens-tubes. This change is produced by the altered ratio of diffusion. A cloudiness of the lens may also be produced in life by a rapid removal of water, as, for example, when a frog is plunged into a salt or sugar solution. The appearance of cloudiness in diabetes has been attributed by some to the removal of water. The views on this subject are, however, contradictory.

The average results of four analyses made by **Laptschinsky**[1] of the lens of oxen are here given, calculated in parts per 1000:

Proteins	349.3
Lecithin	2.3
Cholesterin	2.2
Fat	2.9
Soluble salts	5.3
Insoluble salts	2.3

In cataract the amount of proteins is diminished and the amount of cholesterin increased.

The quantity of the different proteins in the fresh moist lens of oxen is as follows, according to Mörner[2]:

Albumoid (lens-fibres)	170 p. m.
β-Crystallin	110 "
α-Crystallin	68 "
Albumin	2 "

The corneal tissue has been previously considered (page 434). The sclerotic has not been closely investigated, and the choroid coat is chiefly of interest because of the coloring-matter (melanin) it contains (see Chapter XVI).

Tears consist of a water-clear, alkaline fluid of a salty taste. According to the analyses of Lerch[3] they contain 982 p. m. water, 18 p. m. solids with 5 p. m. albumin and 13 p. m. NaCl.

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[1] Pflüger's Arch., 13.

[2] l. c.

[3] Cited from v. Gorup-Besanez, Lehrbuch d. physiol. Chem., 4. Aufl., 401.

THE FLUIDS OF THE INNER EAR.

The **perilymph** and **endolymph** are alkaline fluids which, besides salts, contain—in the same amounts as in transudates—traces of *protein*, and in certain animals (codfish) also *mucin*. The quantity of mucin is greater in the perilymph than in the endolymph.

Otoliths contain 745–795 p. m. inorganic substance, which consists chiefly of crystallized calcium carbonate. The organic substance is very similar to mucin.

CHAPTER XIII.

ORGANS OF GENERATION.

(a) Male Generative Secretions.

THE **testes** have been little investigated chemically. We find in the testes of animals protein bodies of different kinds—*seralbumin, alkali albuminate* (?), and an albuminous body related to ROVIDA's *hyaline substance;* also *leucine, tyrosine, creatine, xanthine bodies, cholesterin, lecithin, inosite,* and *fat.* In regard to the occurrence of glycogen the statements are somewhat contradictory. DARESTE[1] found in the testes of birds starch-like granules, which were colored blue with difficulty by iodine.

In the autolysis of the testes LEVENE[2] found tyrosine, alanine, leucine, aminovalerianic acid, aminobutyric acid, α-proline, phenylalanine, aspartic acid, glutamic acid, and hypoxanthine. Pyrimidine and hexone bases could not be detected.

The **semen** as ejected is a white or whitish-yellow, viscous, sticky fluid of a milky appearance, with whitish, non-transparent lumps. The milky appearance is due to spermatozoa. Semen is heavier than water, contains proteins, has a neutral or faintly alkaline reaction and a peculiar specific odor. Soon after ejection semen becomes gelatinous, as if it were coagulated, but afterwards becomes more fluid. When diluted with water white flakes or shreds separate (HENLE's *fibrin*). According to the analyses of SLOWTZOFF,[3] human semen contains on an average 96.8 p. m. solids with 9 p. m. inorganic and 87.8 p. m. organic substance. The amount of protein substances was on an average, 22.6 p. m. and 1.69 p. m. of bodies soluble in ether. The protein substances consist of *nucleoproteids*, traces of mucin, albumin, and a substance similar to proteose (found earlier by POSNER). According to CAVAZZANI[4] semen contains relatively considerable *nucleon*, more than any organ. The mineral bodies consist chiefly of calcium phosphate and considerable NaCl. Potassium occurs only in smaller amounts.

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[1] Compt. rend., **74**.

[2] Amer. Journ. of Physiol., **11**.

[3] Zeitschr. f. physiol. Chem., **35**.

[4] Posner, Berl. klin. Wochenschr., 1888, No. 21, and Centralbl. f. d. med. Wissensch., 1890; Cavazzani, Biochem. Centralbl., 1, 502, and Centralbl. f. Physiol., **19**.

The semen in the vas deferens differs chiefly from the ejected semen in that it is without the peculiar odor. This last depends on the admixture with the secretion of the prostate. This secretion, according to IVERSEN, has a milky appearance and ordinarily an alkaline reaction, very rarely a neutral one, and contains small amounts of proteins, especially *nucleoproteids*, besides a substance similar to *fibrinogen* and to *mucin* (STERN [1]), and mineral bodies, especially NaCl. Besides this it contains an enzyme *vesiculase* (see below), *lecithin*, *choline* (STERN), and a crystalline combination of phosphoric acid with a base, C_2H_5N. This combination has been called BÖTTCHER's *spermine crystals*, and it is claimed that the specific odor of the semen is due to a partial decomposition of these crystals.

The crystals which appear on slowly evaporating the semen, and which are also observed in anatomical preparations kept in alcohol, are not identical with the CHARCOT-LEYDEN crystals found in the blood and in the lymphatic glands in leucæmia (TH. COHN, B. LEWY [2]). They are, according to SCHREINER,[3] as above stated, a combination of phosphoric acid with a base, *spermine*, C_2H_5N, which he discovered.

Spermine. The views in regard to the nature of this base are not unanimous. According to the investigations of LADENBURG and ABEL, it is not improbable that spermine is identical with ethyleneimine; but this identity is disputed by MAJERT and A. SCHMIDT, and also by POEHL. The compound of spermine with phosphoric acid—BÖTTCHER's spermine crystals—is insoluble in alcohol, ether, and chloroform, soluble with difficulty in cold water, but more readily in hot water, and easily soluble in dilute acids or alkalies, also alkali carbonates and ammonia. The base is precipitated by tannic acid, mercuric chloride, gold chloride, platinic chloride, potassium-bismuth iodide, and phosphotungstic acid. Spermine has a tonic action, and according to POEHL [4] it has a marked action on the oxidation processes of the animal body.

On the addition of a solution of potassium iodide and iodine to spermatozoa, characteristic dark-brown or bluish-black crystals are obtained—FLORENCE'S sperm reaction, which is considered by many as a reaction for spermine. According to BOCARIUS,[5] this reaction is due to choline.

CAMUS and GLEY [6] have found that the prostate fluid in certain rodents has the property of coagulating the contents of the seminal vesicles. This property is due to a special ferment substance (*vesiculase*) of the prostate fluid.

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[1] Iversen, Nord. med. Ark., 6; also Maly's Jahresber., **4**, 358; Stern, Biochem. Centralbl., **1**, 748.

[2] Th. Cohn, Centralbl. f. allg. Path. u. path. Anat., **10** (1899); B. Lewy, Centralbl. f. d. med. Wissensch., 1899, 479.

[3] Annal. d. Chem. u. Pharm., **194**.

[4] Ladenburg and Abel, Ber. d. deutsch. chem. Gesellsch., **21**; Majert and A. Schmidt, *ibid.*, **24**; Poehl, Compt. rend., **115**, Berlin. klin. Wochenschr., 1891 and 1893, Deutsch. med. Wochenschr., 1892 and 1895, and Zeitschr. f. klin. Med., 1894.

[5] In regard to Florence's sperm reaction, see Posner, Berl. klin. Wochenschr. 1897, and Richter, Wien. klin. Wochenschr., 1897; Bocarius, Zeitschr. f. physiol. Chem., **34**.

[6] Compt. rend. de soc. biolog., **48**, **49**.

The spermatozoa show a great resistance to chemical reagents in general. They do not dissolve completely in concentrated sulphuric acid, nitric acid, acetic acid, nor in boiling-hot soda solutions. They are soluble in a boiling-hot caustic-potash solution. They resist putrefaction, and after drying they may be obtained again in their original form by moistening them with a 1 per cent common-salt solution. By careful heating and burning to an ash the shape of the spermatozoa may be seen in the ash. The quantity of ash is about 50 p. m. and consists mainly ($\frac{3}{4}$) of potassium phosphate.

The spermatozoa show well-known movements, but the cause of this is not known. These movements may continue for a very long time, as under some conditions they may be observed for several days in the body after death, and in the secretion of the uterus longer than a week. Acid liquids stop these movements immediately; they are also destroyed by strong alkalies, especially ammoniacal liquids, also by distilled water, alcohol, ether, etc. The movements continue for a longer time in faintly alkaline liquids, especially in alkaline animal secretions, and also in properly diluted neutral salt solutions.

Spermatozoa are nucleus formations and hence are rich in nucleic acid, which exists in the heads. The tails contain protein and are besides this rich in lecithin, cholesterin, and fat, which bodies occur only to a small extent (if at all) in the heads. The tails seem by their composition to be closely allied to the non-medullated nerves or the axis-cylinders. In the various kinds of animals investigated, the head contains nucleic acid, which in fishes is partly combined with protamines and partly with histones. In other animals, such as the bull and boar, protein-like substances occur with the nucleic acid, but no protamine.

Our knowledge of the chemical composition of spermatozoa has been greatly enhanced by the important investigations of MIESCHER[1] on salmon milt. The intermediate fluid of the spermatozoa of Rhine salmon is a dilute salt solution containing 1.3–1.9 p. m. organic and 6.5–7.5 p. m. inorganic bodies. The last consist chiefly of sodium chloride and carbonate, besides some potassium chloride and sulphate. The fluid contains only traces of protein, but no peptone. The tails consist of 419 p. m. protein, 318.3 p. m. lecithin, and 262.7 p. m. cholesterin and fat. The heads extracted with alcohol-ether contain on an average 960 p. m. protamine nucleate, which nevertheless is not uniform, but is so divided that the outer layers consist of basic protamine nucleate, while the inner layers, on the contrary, consist of acid protamine nucleate. Besides the protamine nucleate there are present in the heads, although to a very slight extent, unknown organic substances. The unripe salmon spermatozoa, while developing, also contain nucleic acid, but no protamine, with a protein substance, "*albuminose*," which probably is a step in the formation of protamine. According to KOSSEL and MATHEWS,[1] in the herring as in the salmon, the heads of the spermatozoa consist of protamine nucleate but no free protein.

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[1] See Miescher, "Die histochemischen und physiologischen Arbeiten von Friedrich Miescher, gesammelt und herausgegeben von seinen Freunden," Leipzig, 1897.

Spermatin is a name which has been given to a constituent similar to alkali albuminate, but it has not been closely studied.

Prostatic concrements are of two kinds. One is very small, generally oval in shape, with concentric layers. In young but not in older persons they are colored blue by iodine (IVERSEN[2]). The other kind is larger, sometimes the size of the head of a pin, and consisting chiefly of calcium phosphate (about 700 p. m.), with only a very small amount (about 160 p. m.) of organic substance.

(b) Female Generative Organs.

The stroma of the **ovaries** is of little interest from a physiologico-chemical standpoint, and the most important constituents of the ovaries, the *Graafian follicles* with the *ovum*, have not thus far been the subject of a careful chemical investigation. The fluid in the follicles (of the cow) does not contain, as has been stated, the peculiar bodies, paralbumin or metalbumin, which are found in certain pathological ovarial fluids, but seems to be a serous liquid. The *corpora lutea* are colored yellow by an amorphous pigment called *lutein*. Besides this another coloring-matter sometimes occurs which is not soluble in alkali; it is crystalline, but not identical with bilirubin or hæmatoidin; but it may be identified as a lutein by its spectroscopic behavior (PICCOLO and LIEBEN, KÜHNE and EWALD[3]).

The cysts often occurring in the ovaries are of special pathological interest, and these may have essentially different contents, depending upon their variety and origin.

The **serous cysts** (HYDROPS FOLLICULORUM GRAAFII), which are formed by a dilation of the Graafian follicles, contain a serous liquid which has a specific gravity of 1.005–1.022. A specific gravity of 1.020 is less frequent. Generally the specific gravity is lower, 1.005–1.014, with 10–40 p. m. solids. As far as is known, the contents of these cysts do not essentially differ from other serous liquids.

The **proliferous cysts** (MYXOID CYSTS, COLLOID CYSTS), which are developed from PFLÜGER's epithelium-tubes, may have a content of a decidedly variable composition.

We sometimes find in small cysts a semi-solid, transparent, or somewhat cloudy or opalescent mass which appears like solidified glue or quivering jelly, and which has been called *colloid* because of its physical properties. In other cases the cysts contain a thick, tough mass which can be drawn out into long threads, and as this mass in the different cysts is more or less diluted with serous liquids their contents may have a variable consistency. In still other cases the small cysts may also contain a thin, watery fluid. The color of the contents is also variable. Sometimes they are bluish white, opalescent, and again they are yellow, yellowish brown, or yellowish with a shade of green. They are often colored more or less chocolate-brown or red-brown, due to the decomposed blood-coloring matters. The reaction is alkaline or nearly neutral. The specific gravity, which may vary considerably, is generally 1.015–1.030, but may occasionally be 1.005–1.010 or 1.050–1.055. The amount of solids is very variable. In rare cases it amounts to only 10–20 p. m.; ordinarily it varies between 50–70–100 p. m. In a few instances 150–200 p. m. solids have been found.

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[1] Zeitschr. f. physiol. Chem., 23. [2] Nord. med. Ark., 6. [3] See Chapter VI, p. 216.

As form-elements one finds red and white *blood-corpuscles*, *granular cells*, partly fat-degenerated epithelium and partly large so-called GLUGE's corpuscles, *fine granular masses*, *epithelium-cells*, *cholesterin crystals*, and *colloid corpuscles*—large, circular, highly refractive formations.

Though the contents of the proliferous cyst may have a variable composition, still it may be characterized in typical cases by its slimy or ropy consistency; by its grayish-yellow, chocolate-brown, sometimes whitish-gray color; and by its relatively high specific gravity, 1.015–1.025. Such a liquid does not ordinarily show a spontaneous fibrin coagulation.

We consider *colloid*, *metalbumin*, and *paralbumin* as characteristic constituents of these cysts.

Colloid. This name does not designate any particular chemical substance, but is given to the contents of tumors with certain physical properties similar to gelatine jelly. Colloid is found as a pathological product in several organs.

Colloid is a gelatinous mass, insoluble in water and acetic acid; it is dissolved by alkalies and gives a liquid which is not precipitated by acetic acid or by acetic acid and potassium ferrocyanide. According to PFANNENSTIEL[1] such a colloid is designated β-pseudomucin. Sometimes a colloid is found which, when treated with a very dilute alkali, gives a solution similar to a mucin solution. Colloid is very closely related to mucin and is considered by certain investigators as a modified mucin. An ovarial colloid analyzed by PANZER contained 931 p. m. water, 57 p. m. organic substance, and 12 p. m. ash. The elementary composition was C 47.27, H 5.86, N 8.40, S 0.79, P 0.54, and ash 6.43 per cent. A colloid found by WURTZ[2] in the lungs contained C 48.09, H 7.47, N 7.00, and O(+S) 37.44 per cent. Colloids of different origin seem to be of varying composition.

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[1] Arch. f. Gynäk., 38.

[2] Panzer, Zeitschr. f. physiol. Chem., 28; Wurtz, see Lebert, Beitr. zur Kenntnis des Gallertkrebses, Virchow's Arch., 4.

Metalbumin. This name Scherer[1] gave to a protein substance found by him in an ovarial fluid. The metalbumin was considered by Scherer to be an albuminous body, but it belongs to the mucin group, and it is for this reason called *pseudomucin* by Hammarsten.[2]

Pseudomucin. This body, which, like the mucins, gives a reducing substance when boiled with acids, is a mucoid of the following composition: C 49.75, H 6.98, N 10.28, S 1.25, O 31.74 per cent (Hammarsten). With water pseudomucin gives a slimy, ropy solution, and it is this substance which gives the fluid contents of the ovarial cysts their typical ropy property. Its solutions do not coagulate on boiling, but only become milky or opalescent. Unlike mucin, pseudomucin solutions are not precipitated by acetic acid. With alcohol they give a coarse flocculent or thready precipitate which is soluble even after having been kept under water or alcohol for a long time.

Paralbumin is another substance discovered by Scherer, which occurs in ovarial liquids and also in ascitic fluids with the simultaneous presence of ovarial cysts and rupture of the same. It is therefore only a mixture of pseudomucin with variable amounts of protein, and the reactions of paralbumin are correspondingly variable.

Mitjukoff[3] has isolated and investigated a colloid from an ovarial cyst. It had the following composition: C 51.76, H 7.76, N 10.7, S 1.09, and O 28.69 per cent, and differed from mucin and pseudomucin by reducing Fehling's solution before boiling with acid. It must be remarked that pseudomucin, on boiling sufficiently long with alkali, or by the use of a concentrated solution of caustic alkali, also splits and causes a reduction. This reduction is nevertheless weak as compared with that produced after boiling with an acid. The body isolated by Mitjukoff is called *paramucin.*

The pseudomucin as well as colloid are mucoid substances, and the carbohydrate obtained from them is glucosamine (chitosamine), as especially shown by Fr. Müller, Neuberg and Heymann.[4] From pseudomucin Zängerle[5] obtained 30 per cent glucosamine, and Neuberg and Heymann have shown that the glucosamine is the only carbohydrate regularly taking part in the structure of these substances. Still there are also statements as to the occurrence of chondroitin-sulphuric acid (or an allied acid) in pseudomucin or colloid (Panzer), but this is not constant according to the experience of Hammarsten.

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[1] Verh. d. physik.-med. Gesellsch. in Würzburg, **2**, and Sitzungsber. der physik.-med. Gesellsch. in Würzburg für 1864-1865; Würzburg med. Zeitschr., **7**, No. 6.

[2] Zeitschr. f. physiol. Chem., **6**.

[3] K. Mitjukoff, Arch. f. Gynäkol., **49**.

[4] Müller, Verh. d. Naturf. Gesellsch. in Basel, **12**, part 2; Neuberg and Heymann, Hofmeister's Beiträge, **2**. See also Leathes, Arch. f. exp. Path. u. Pharm., **43**.

[5] Münch. med. Wochenschr., 1900.

As hydrolytic cleavage products of pseudomucin OTORI[1] has obtained, besides carbohydrate derivatives such as levulinic acid and humus substances, leucine, tyrosine, glycocoll, aspartic acid, glutamic acid, valerianic acid, arginine, lysine, and guanidine. The quantity of guanidine, it seems, was greater than that which could be derived from the arginine, hence this body probably originated from another complex.

The detection of metalbumin and paralbumin is naturally connected with the detection of pseudomucin. A typical ovarial fluid containing pseudomucin is, as a rule, sufficiently characterized by its physical properties, and a special chemical investigation is necessary only in cases where a serous fluid contains very small amounts of pseudomucin. The procedure is as follows: The protein is removed by heating to boiling with the addition of acetic acid; the filtrate is strongly concentrated and precipitated by alcohol. The precipitate, a transformation product of pseudomucin, is carefully washed with alcohol and then dissolved in water. A part of this solution is digested with saliva at the temperature of the body and then tested for glucose (derived from glycogen or dextrin). If glycogen is present, it will be converted into glucose by the saliva; precipitate again with alcohol and then proceed as in the absence of glycogen. In this last-mentioned case, first add acetic acid to the solution of the alcohol precipitate in water so as to precipitate any existing mucin. The precipitate produced is filtered off, the filtrate treated with 2 per cent HCl and warmed on the water-bath until the liquid is deep brown in color. In the presence of pseudomucin this solution gives TROMMER'S test.

The other protein bodies which have been found in cystic fluids are *serglobulin* and *seralbumin*, *peptone* (?), *mucin*, and *mucin-peptone* (?). Fibrin occurs only in exceptional cases. The quantity of mineral bodies on an average amounts to about 10 p. m. The amount of extractive bodies (*cholesterin* and *urea*) and *fat* is ordinarily 2–4 p. m. The remaining solids, which constitute the chief mass, are protein bodies and pseudomucin.

The **intraligamentary, papillary cysts** contain a yellow, yellowish-green, or brownish-green liquid which contains either no pseudomucin or very little. The specific gravity is generally rather high, 1.032–1.036, with 90–100 p. m. solids. The principal constituents are the simple proteins of blood-serum.

The rare *tubo-ovarial cysts* contain as a rule a watery, serous fluid containing no pseudomucin.

The *parovarial cysts* or the CYSTS of the LIGAMENTA LATA may attain a considerable size. In general, and when quite typical, the contents are watery, mostly very pale yellow-colored, water-clear or only slightly opalescent liquids. The specific gravity is low, 1.002–1.009, and the solids only amount to 10–20 p. m. Pseudomucin does not occur as a typical constituent; protein is sometimes absent, and when it does occur the quantity is very small. The principal part of the solids consists of salts and extractive bodies. In exceptional cases the fluid may be rich in protein and may show a higher specific gravity.

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[1] Zeitschr. f. physiol. Chem., 42 and 43.

In regard to the quantitative composition of the fluid from ovarial cysts we refer the reader to the work of OERUM.[1]

E. LUDWIG and R. v. ZEYNEK[2] have recently investigated the fat from dermoid cysts. Besides a little arachidic acid, they found oleic, stearic, palmitic, and myristic acids, cetyl alcohol, and a cholesterin-like substance.

The colloid from a uterine fibroma analyzed by STOLLMANN[3] contained a pseudomucin soluble in water and a colloid (paramucin) insoluble in water, both of which behaved differently with alcohol as compared with the corresponding substances from ovarial cysts.

The Ovum.

The small ova of man and mammals cannot, for evident reasons, be the subject of a searching chemical investigation. Up to the present time the eggs of birds, amphibians, and fishes have been investigated, but above all the hen's egg. We will here occupy ourselves with the constituents of this last.

The Yolk of the Hen's Egg. In the so-called white yolk, which forms the *germ* with a process reaching to the centre of the yolk (*latebra*), and forming a layer between the yolk and yolk-membrane, there occur *protein, nuclein, lecithin,* and *potassium* (LIEBERMANN[4]). The occurrence of glycogen is doubtful. The yolk-membrane consists of an albuminoid similar in certain respects to keratin (LIEBERMANN).

The principal part of the yolk—the nutritive yolk or yellow—is a viscous, non-transparent, pale-yellow or orange-yellow alkaline emulsion of a mild taste. The yolk contains *vitellin, lecithin, cholesterin, fat, coloring-matters,* traces of *neuridine* (BRIEGER[5]), *purine bases* (MESERNITZKI[6]), *glucose* in very small quantities, and *mineral bodies.* The occurrence of cerebrin and of granules similar to starch (DARESTE[7]) has not been positively proved.

Several enzymes have been found in the yolk, especially a diastatic enzyme (MÜLLER and MASUYAMA), a glycolytic enzyme (STEPANEK) which in the absence of air brings about an alcoholic fermentation of sugar and in the presence of air forms carbon dioxide and lactic acid, and finally a proteolytic (WOHLGEMUTH), a lipolytic, and a chromolytic (?) enzyme.[1]

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[1] Kemiske Studier over Ovariecystevædsker, etc., Koebenhavn, 1884. See also Maly's Jahresber., **14**, 459.

[2] Zeitschr. f. physiol. Chem., **23**.

[3] American Gynecology, March, 1903.

[4] Pflüger's Arch., **43**.

[5] Ueber Ptomaïne, Berlin, 1885.

[6] Mesernitzki, Biochem. Centralbl., **1**, 739.

[7] Compt. rend., **72**.

Ovovitellin. This body, which is generally considered as a globulin, is in reality a nucleoalbumin. The question as to what relationship other protein substances which are related to ovovitellin, like the *aleuron grains* of certain seeds and the *yolk spherules* of the eggs of certain fishes and amphibians, bear to this substance is one which requires further investigation.

The ovovitellin which has been prepared from the yolk of eggs is not a pure protein body, but always contains lecithin. HOPPE-SEYLER found 25 per cent lecithin in vitellin. The lecithin may be removed by boiling alcohol, but the vitellin is changed thereby, and it is therefore probable that the lecithin is chemically united with the vitellin (HOPPE-SEYLER[2]). According to OSBORNE and CAMPBELL, the so-called ovovitellin is a mixture of various vitellin-lecithin combinations, with 15–30 per cent of lecithin. The protein substance freed from lecithin is the same in all these compounds and has the following composition: C 51.24, H 7.16, N 16.38, S 1.04, P 0.94, O 23.24 per cent. These figures differ somewhat from those obtained by GROSS[3] for vitellin prepared by another method (precipitation with $(NH_4)_2SO_4$), namely, C 48.01, H 6.35, N 14.91-16.97, P 0.32–0.35, S 0.88, and the composition of ovovitellin is therefore not positively known. GROSS found in vitellin a globulin coagulating at 76–77° C. in a solution containing hydrochloric acid.

On the pepsin digestion of ovovitellin, OSBORNE and CAMPBELL obtained a pseudonuclein with varying amounts of phosphorus, 2.52–4.19 per cent. BUNGE[4] prepared a pseudonuclein by digesting the yolk with gastric juice, and his pseudonuclein, according to him, is of great importance in the formation of the blood, and on these grounds he called it *hæmatogen*. This hæmatogen has the following composition: C 42.11, H 6.08, N 14.73, S 0.55, P 5.19, Fe 0.29, and O 31.05 per cent. The composition of this substance may vary considerably even on using the same method of preparation.

Vitellin is similar to the globulins in that it is insoluble in water, but on the contrary soluble in dilute neutral-salt solutions (although the solution is not quite transparent). It is also soluble in hydrochloric acid of 1 p. m. and in very dilute solutions of alkalies or alkali carbonates. It is precipitated from its salt solution by diluting with water, and when allowed to stand some time in contact with water the vitellin is gradually changed, forming a substance more like the albuminates. The coagulation temperature for the solution containing salt (NaCl) lies between 70° and 75° C., or, when heated very rapidly, at about 80° C. Vitellin differs from the globulins in yielding pseudonuclein by peptic digestion. It is not always completely precipitated by NaCl in substance. The ovovitellin isolated by GROSS gave MOLISCH's reaction. NEUBERG[1] has also split off glucosamine from the yolk and has identified it as norisosaccharic acid. It is difficult to state whether this glucosamine was derived from the vitellin or from some other constituent of the yolk.

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[1] Müller and Masuyama, Zeitschr. f. Biologie, **39**; Stepanek, Centralbl. f. Physiol., **18**, 188; Wohlgemuth in Salkowski's Festschrift and Zeitschr. f. physiol. Chem., **44**.

[2] Med. chem. Untersuch. 216.

[3] Osborne and Campbell, Connecticut Agric. Exp. Station, 23d Ann. Report, New Haven, 1900; Gross, Zur Kenntn. d. Ovovitellins, Inaug.-Diss. Strassburg, 1899.

[4] Zeitschr. f. physiol. Chem., **9**, 49. See also Hugounenq and Morel, Compt. rend., **140** and **141**.

The chief points in the preparation of ovovitellin are as follows: The yolk is thoroughly agitated with ether; the residue is dissolved in a 10 per cent common-salt solution, filtered, and the vitellin precipitated by adding an abundance of water. The vitellin is now purified by repeatedly redissolving in dilute common-salt solutions and precipitating with water.

Ichthulin, which occurs in the eggs of the carp and other fishes, is, according to KOSSEL and WALTER, an amorphous modification of the crystalline body *ichthidin*, which occurs in the eggs of the carp. Ichthulin is precipitated on diluting with water. It used to be considered as a vitellin. According to WALTER it yields a pseudonuclein on peptic digestion; and this pseudonuclein gives a reducing carbohydrate on boiling with sulphuric acid. Ichthulin has the following composition: C 53.42, H 7.63, N 15.63, O 22.19, S 0.41, P 0.43. It also contains iron. The ichthulin investigated by LEVENE from codfish eggs had the composition C 52.44, H 7.45, N 15.96, S 0.92, P 0.65, Fe+O 22.58 per cent, and yielded no reducing substances on boiling with acids. The pure vitellin isolated by HAMMARSTEN[2] from perch eggs had a similar behavior and was very readily changed by a little hydrochloric acid so that it was converted into a typical pseudonuclein. The codfish ichthulin yielded a pseudonucleic acid with 10.34 per cent phosphorus, but this acid still gave the protein reactions.

The yolk also contains *albumin*, besides vitellin and the above-mentioned globulin.

The *fat* of the yolk of the egg is, according to LIEBERMANN, a mixture of a solid and a liquid fat. The solid fat consists chiefly of tripalmitin with some tristearin. On the saponification of the egg-oil LIEBERMANN obtained 40 per cent oleic acid, 38.04 per cent palmitic acid, and 15.21 per cent stearic acid. The fat of the yolk of the egg contains less carbon than other fats, which may depend upon the presence of monoglycerides and diglycerides, or upon a quantity of fatty acid deficient in carbon (LIEBERMANN). In the lecithin, or more correctly in the lecithin mixture of the yolk, COUSIN finds also linolic acid besides the three ordinary fatty acids. The composition of yolk fat is dependent upon the food, as HENRIQUES and HANSEN[3] have shown that the fat of the food passes into the egg.

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[1] Ber. d. d. chem. Gesellsch., **34**.

[2] Walter, Zeitschr. f. physiol. Chem., **15**; Levene, *ibid.*, **32**; Hammarsten, Skand. Arch. f. Physiol., **17**.

[3] Cousin, Compt. rend., **137**; Henriques and Hansen, Skand. Arch. f. Physiol., **14**.

Lutein. Yellow or orange-red amorphous coloring-matters occur in the yellow of the egg and in several other places in the animal organism; for instance, in the blood-serum and serous fluids, fatty tissues, milk-fat, *corpora lutea*, and in the fat-globules of the retina. These coloring-matters, which also occur in the vegetable kingdom (THUDICHUM), and whose relationship to the vegetable pigments, the xanthophyll group, has recently been shown by SCHUNCK,[1] have been called *luteins* or *lipochromes*.

The luteins, which among themselves show somewhat different properties, are all soluble in alcohol, ether, and chloroform. They differ from the bile-pigment, bilirubin, in that they are not separated from their solution in chloroform by water containing alkali, and also in that they do not give the characteristic play of colors with nitric acid containing a little nitrous acid, but give a transient blue color, and, lastly, they ordinarily show an absorption-spectrum of two bands, of which one covers the line *F* and the other lies between the lines *F* and *G*. The luteins withstand the action of alkalies so that they are not changed when we remove the fats present by means of saponification.

Lutein has not been prepared pure. MALY[2] has found two pigments free from iron in the eggs of a water-spider (Maja squinado)—one a red (*vitellorubin*) and the other a yellow pigment (*vitellolutein*). Both of these pigments are colored blue by nitric acid containing nitrous acid and beautifully green by concentrated sulphuric acid. The absorption-bands, especially of the vitellolutein, correspond very nearly to those of ovolutein.

The *mineral bodies* of the yolk of the egg consist, according to POLECK,[3] of 51.2–65.7 parts soda, 80.5–89.3 potash, 122.1–132.8 lime, 20.7–21.1 magnesia, 11.90–14.5 iron oxide, 638.1–667.0 phosphoric acid, and 5.5–14.0 parts silicic acid in 1000 parts of the ash. We find phosphoric acid and lime the most abundant, and then potash, which is somewhat greater in quantity than the soda. These results are not, however, quite correct: first, because no dissolved phosphate occurs in the yolk (LIEBERMANN), and secondly, in burning, phosphoric and sulphuric acids are produced, and these drive away the chlorine, which is not accounted for in the preceding analyses.

The yolk of the hen's egg weighs about 12–18 grams. The quantity of water and solids amounts, according to PARKE,[4] to 471.9 p. m. and 528.1 p. m. respectively. Among the solids he found 156.3 p. m. protein, 3.53 p. m. soluble and 6.12 p. m. insoluble salts. The quantity of fat, according to PARKE, is 228.4 p. m.; the lecithin, calculated from the amount of phosphorus in the organic substance of the alcohol-ether extract, was 107.2 p. m. and the cholesterin 17.5 p. m.

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[1] Thudichum, Centralbl. f. d. med. Wissensch., 1869; Schunck, see Chem. Centralbl., 1903, 2, 1195.

[2] Monatshefte f. Chem., 2.

[3] Cited from v. Gorup-Besanez, Lehrbuch d. physiol. Chem., 4. Aufl., 740.

[4] Hoppe-Seyler, Med. chem. Untersuch., Heft 2, 209.

The **white of the egg** is a faintly yellow albuminous fluid inclosed in a framework of thin membranes; and this fluid is in itself very liquid, but seems viscous because of the presence of these fine membranes. That substance which forms the membranes, and of which the *chalaza* consists, seems to be a body nearly related to horn substances (LIEBERMANN).

The white of the egg has a specific gravity of 1.045 and always has an alkaline reaction towards litmus. It contains 850–880 p. m. water, 100–130 p. m. protein bodies, and 7 p. m. salts. Among the extractive bodies LEHMANN found a fermentable *variety of sugar* which amounted to 5 p. m. or, according to MEISSNER, 80 p. m. of the solids.[1] Besides these one finds in the white of the egg traces of fats, soaps, lecithin and cholesterin.

The white of the egg of the Insessores becomes transparent on boiling and acts in many respects like alkali albuminate. This albumin TARCHANOFF[2] called "*tatalbumin*."

The protein substances of the white of egg are all glucoproteids, as they all yield glucosamine. According to the solution and precipitation properties they are similar to the globulins, albumins or proteoses. The representatives of the first two groups, which until recently were considered as true proteins, are *ovoglobulin* and *ovalbumin*. The proteose-like body is *ovomucoid*.

Ovoglobulin separates in part on diluting the egg-white with water. It is precipitated upon saturation with magnesium sulphate or upon one-half saturation with ammonium sulphate and coagulates at about 75° C. By repeated solution in water and precipitation with ammonium sulphate a part of the globulin becomes insoluble (LANGSTEIN). This also occurs on precipitation by diluting with water or by dialysis, and it is quite possible that the globulin is a mixture. That portion which readily becomes insoluble seems to be identical with EICHHOLZ's glucoproteid or OSBORNE and CAMPBELL's ovomucin. LANGSTEIN obtained 11 per cent of glucosamine from the soluble ovoglobulin. The total quantity of globulins, according to DILLNER, is about 6.7 per cent of the total protein substances, and this corresponds with the recent determinations of OSBORNE and CAMPBELL. In regard to the probable occurrence of several globulins in the white of the egg there are the statements of CORIN and BERARD as well as of LANGSTEIN,[3] but they have not led to any positive conclusions.

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[1] Cited from v. Gorup-Besanez, Lehrbuch, 4. Aufl., 739.

[2] Pflüger's Arch., **31**, **33**, and **39**.

[3] Langstein, Hofmeister's Beiträge, **1**; Eichholz, Journ. of Physiol., **23**; Osborne and Campbell, Connecticut Agric. Exp. Station, 23d Ann. Report, New Haven, 1900; Dillner, Maly's Jahresber., **15**; Corin and Berard, *ibid.*, **18**.

Ovalbumin. The so-called albumin of the egg-white is undoubtedly a mixture of at least two albumin-like glucoproteids. The views differ considerably in regard to the number of these compound proteids (BONDZYNSKI and ZOJA, GAUTIER, BÉCHAMP, CORIN and BERARD, PANORMOFF, and others). Since HOFMEISTER has been able to prepare ovalbumin in a crystalline form, and since HOPKINS and PINKUS[1] have shown that not more than one-half of the ovalbumin can be obtained in such a form, OSBORNE and CAMPBELL have isolated two different ovalbumins or chief fractions; the crystallizable they call *ovalbumin* and the non-crystallizable *conalbumin*. The two fractions have only a slight variation in elementary composition; the conalbumin coagulates between 50–60° C., nearer to 60° C., and the ovalbumin at 64° C. or at a higher temperature. There are no conclusive investigations as to whether the non-crystallizable conalbumin is a mixture or not, and the question concerning the unity of the crystallizable ovalbumin is also disputed. According to BONDZYNSKI and ZOJA, crystallizable ovalbumin is a mixture of several albumins having somewhat different coagulation temperatures, solubilities, and specific rotations, while HOFMEISTER and LANGSTEIN on the contrary believe that crystallizable ovalbumin is a unit. The statements as to the specific rotation of the different fractions unfortunately differ, and the elementary analyses have also given no positive results, as a variation of 1.2–1.7 per cent has been observed in the quantity of sulphur. According to the consistent analyses of OSBORNE and CAMPBELL and of LANGSTEIN, the conalbumin contains about 1.7 per cent sulphur and about 16 per cent nitrogen, while the ovalbumin contains on an average about 15.3 per cent nitrogen. LANGSTEIN[2] obtained 10–11 per cent glucosamine from ovalbumin and about 9 per cent from conalbumin. The ovalbumin, like the conalbumin, has the properties of the albumins in general, but differs from seralbumin in the following: The specific rotation is lower. It is made quickly insoluble by alcohol and is precipitated by a sufficient quantity of HCl, but dissolves in an excess of acid with greater difficulty than the seralbumin. The products isolated by ABDERHALDEN and PREGL[3] on the hydrolysis of ovalbumin do not show anything of special interest.

In preparing crystalline ovalbumin mix, according to HOFMEISTER, the beaten white of egg free from foam with an equal volume of a saturated ammonium-sulphate solution, filter off the globulin, and allow the filtrate to slowly evaporate in thin layers at the temperature of the room. After a time the masses which separate out are dissolved in water, treated with ammonium-sulphate solution until they begin to get cloudy, and allowed to stand. After repeated recrystallization the mass is either treated with alcohol, which makes the crystals insoluble, or they are dissolved in water and purified by dialysis. From these solutions the proteid does not crystallize again on spontaneous evaporation. (See also page 507, foot-note 1, for the HOPKINS and PINKUS method.)

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[1] Hofmeister, Zeitschr. f. physiol. Chem., **14**, **16**, and **24**; Gabriel, *ibid.*, **15**; Bondzynski and Zoja, *ibid.*, **19**; Gautier, Bull. Soc. chim., **14**; Béchamp, *ibid.*, **21**; Corin and Berard, l. c.; Hopkins and Pinkus, Ber. d. d. chem. Gesellsch., **31**, and Journ. of Physiol., **23**; Osborne and Campbell, l. c.; Panormoff, Maly s Jahresber., **27** and **28**.

[2] Zeitschr. f. physiol. Chem., **31**.

[3] *Ibid.*, **46**.

Conalbumin can be removed from the filtrate, after the complete crystallization of the ovalbumin, by removing the sulphate by means of dialysis and coagulating by heat.

GAUTIER [1] found a fibrinogen-like substance in the white of the egg, which was changed into a fibrin-like body by the action of a ferment.

Ovomucoid. This substance, first observed by NEUMEISTER and considered by him as a pseudopeptone and then later studied by SALKOWSKI, is, according to C. TH. MÖRNER,[2] a mucoid with 12.65 per cent nitrogen and 2.20 per cent sulphur. On boiling with dilute mineral acids it yields a reducing substance. Ovomucoid exists in hens' eggs to the extent of about 10 per cent of the total solids.

A solution of ovomucoid is not precipitated by mineral acids nor by organic acids, with the exception of phosphotungstic acid and tannic acid. It is not precipitated by metallic salts, but basic lead acetate and ammonia render it insoluble. Ovomucoid is thrown down by alcohol, but sodium chloride, sodium sulphate, and magnesium sulphate give no precipitates either at the ordinary temperature or when the salts are added to saturation at 30° C. Its solutions are not precipitated by an equal volume of a saturated solution of ammonium sulphate, but are precipitated on adding more salt thereto. The substance is not precipitated on boiling, but the part which has become insoluble in cold water which has been dried is dissolved by boiling water. ZANETTI has prepared glucosamine on splitting ovomucoid with concentrated hydrochloric acid, and SEEMANN found that the quantity of glucosamine in ovomucoid was 34.9 per cent.[3]

Ovomucoid may be prepared by removing all the proteins by boiling with the addition of acetic acid and then concentrating the filtrate and precipitating with alcohol. The substance is purified by repeated solution in water and precipitation with alcohol.

According to PANORMOW [4] the eggs of other birds, such as the pigeon and ducks, contain a special protein in the egg-white, which is not identical with that of the hen's egg.

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[1] Compt. rend., **135**.

[2] R. Neumeister, Zeitschr. f. Biologie, **27**; Salkowski, Centralbl. f. d. med. Wissensch., 1893, 513 and 706; C. Mörner, Zeitschr. f. physiol. Chem., **18**. See also Langstein, Hofmeister's Beiträge, **3** (literature).

[3] Zanetti, Chem. Centralbl., 1898, **1**; Seemann, cited from Langstein, Ergebnisse der Physiol., **1**, Abt. 1, 86.

[4] See Biochem. Centralbl. **5**

The *mineral bodies* of the white of the egg have been analyzed by POLECK and WEBER.[1] They found in 1000 parts of the ash: 276.6–284.5 grams potash, 235.6–329.3 soda, 17.4–29 lime, 17–31.7 magnesia, 4.4–5.5 iron oxide, 238.4–285.6 chlorine, 31.6–48.3 phosphoric acid (P_2O_5), 13.2–26.3 sulphuric acid, 2.8–20.4 silicic acid, and 96.7–116 grams carbon dioxide. Traces of fluorine have also been found (NICKLÈS [2]). The ash of the white of the egg contains, as compared with the yolk, a greater amount of chlorine and alkalies and a smaller amount of lime, phosphoric acid, and iron.

The Shell-membrane and the Egg-shell. The shell-membrane consists, as above stated (page 73), of a keratin substance. The shell contains very little organic substance, 36–65 p. m. The chief mass, more than 900 p. m., consists of calcium carbonate; besides this there are very small amounts of magnesium carbonate and earthy phosphates.

The diverse *coloring* of birds' eggs is due to several different coloring-matters. Among these we find a red or reddish-brown pigment called "*oorodein*" by SORBY,[3] which is perhaps identical with hæmatoporphyrin. The green or blue coloring-matter, SORBY'S *oocyan*, seems, according to LIEBERMANN [4] and KRUKENBERG,[5] to be partly *biliverdin* and partly a blue *derivative of the bile-pigments.*

The eggs of birds have a space at their blunt end filled with gas; this gas contains on an average 18.0–19.9 per cent oxygen (HÜFNER [6]).

The weight of a hen's egg varies between 40–60 grams and may sometimes reach 70 grams. The shell and shell-membrane together, when carefully cleaned, but still in the moist state, weigh 5–8 grams. The yolk weighs 12–18 and the white 23–34 grams, or about double. The entire egg contains 2.8–7.5, or average 4.6, milligrams of iron oxide, and the quantity of iron can be increased by food rich in iron (HARTUNG [7]).

The white of the egg of cartilaginous and bony fishes contains only traces of true albumin, and the cover of the frog's egg consists, according to GIACOSA, of mucin. The eggs of the river-perch contain, according to HAMMARSTEN,[8] mucin in the envelope in the unripe state and only mucinogen in the ripe state. The crystalline formations (*yolk-spherules*, or *dotterplättchen*) which have been observed in the egg of the tortoise, frog, ray, shark, and other fishes, and which are described by VALENCIENNES and FRÉMY [9] under the names *emydin, ichthin, ichthidin,* and *ichthulin*, seem, as above stated in connection with ichthulin, to consist chiefly of phosphoglucoproteids. The eggs of the river-crab and the lobster contain the same pigment as the shell of the animal. This pigment, called *cyanocrystallin*, becomes red on boiling in water.

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[1] Cited from Hoppe-Seyler, Physiol. Chem., 778.

[2] Compt. rend., 43.

[3] Cited from Krukenberg, Verh. d. phys.-chem. Gesellsch. in Würzburg, 17.

[4] Ber. d. deutsch. chem. Gesellsch., 11.

[5] l. c.

[6] Arch. f. (Anat. u.) Physiol., 1892.

[7] Zeitschr. f. Biologie, 43.

[8] Giacosa, Zeitschr. f. physiol. Chem., 7; Hammarsten, Skand. Arch. f. Physiol., 17.

[9] Cited from Hoppe-Seyler's Physiol. Chem., 77.

C. Morner[1] has isolated a substance which he calls *percaglobulin* from the unripe eggs of the river-perch. It is a globulin and has a strong astringent taste. Especially striking is its property of precipitating certain glucoproteids, such as ovomucoid and ovarial mucoids, and polysaccharides, such as glycogen, gum tragacanth or quince-seed gum, and starch-paste, and of being precipitated by them.

In fossil eggs (of APTENODYTES, PELECANUS, and HALLÆUS) in old guano deposits, a yellowish white, silky, laminated compound has been found which is called *guanovulit*, $(NH_4)_2SO_4+2K_2SO_4+3KHSO_4+4H_2O$, and which is easily soluble in water, but is insoluble in alcohol and ether.

Those eggs which develop outside of the mother-organism must contain all the elements necessary for the young animals. One finds, therefore, in the yolk and white of the egg an abundant quantity of protein bodies of different kinds, and especially phosphorized proteins in the yolk. Further, we also find lecithin in the yolk, which seems habitually to occur in the developing cell. The occurrence of glycogen is doubtful, and the carbohydrates are perhaps represented by a very small amount of sugar and glucoproteids. On the contrary, the egg contains a large proportion of fat, which doubtless is important as a source of supply of nourishment and in maintaining respiration for the embryo. The cholesterin and the lutein can hardly have a direct influence on the development of the embryo. The egg also seems to contain the mineral bodies necessary for the development of the young animal. The lack of phosphoric acid is compensated by an abundant amount of phosphorized organic substance, and the nucleoalbumin containing iron, from which the hæmatogen (see page 503) is formed, is doubtless, as Bunge claims, of great importance in the formation of the hæmoglobin containing iron. The silicic acid necessary for the development of the feathers is also found in the egg.

During the period of incubation the egg loses weight, chiefly due to loss of water. The quantity of solids, especially the fat and the proteins, diminishes, and the egg gives off not only carbon dioxide, but also, as Liebermann[2] has shown, nitrogen or a nitrogenous substance. The loss is compensated by the absorption of oxygen, and it is found that during incubation a respiratory exchange of gases takes place.

As Bohr and Hasselbalch have shown by exact investigations, the elimination of carbon dioxide is very small in the first days of incubation; on the fourth day the carbon-dioxide production gradually increases, and after the ninth day it augments in the same proportion as the weight of the fœtus. Calculated upon 1 kilogram weight for one hour it is, from the ninth day on, about the same as in the full-grown hen. Hasselbalch[3] has also shown that the fertilized hen's egg not only gives off nitrogen the first five or six hours of incubation, but also some oxygen, and that we are here dealing with an oxygen production which runs parallel with the cell-division. It is not known whether this oxygen formation connected with the life of the cell is a fermentative or a so-called vital process.

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[1] Zeitschr. f. physiol. Chem., **40**.

[2] Pflüger's Arch., **43**.

[3] Bohr and Hasselbalch, Maly's Jahresber., **29**; Hasselbalch, Skand. Arch. f. Physiol., **13**.

While the quantity of dry substance in the egg during this period always decreases, the quantity of mineral bodies, protein, and fat always increases in the embryo. The increase in the amount of fat in the embryo depends, according to LIEBERMANN, in great part upon a taking up of the nutritive yolk in the abdominal cavity. The weight of the shell and the quantity of lime-salts contained therein remain unchanged during incubation. The yolk and white together contain the necessary quantity of lime for development.

The most complete and careful chemical investigation on the development of the embryo of the hen has been made by LIEBERMANN. From his researches we may quote the following: In the earlier stages of the development, tissues very rich in water are formed, but upon the continuation of the development the quantity of water decreases. The absolute quantity of the bodies soluble in water increases with the development, while their relative quantity, as compared with the other solids, continually decreases. The quantity of the bodies soluble in alcohol quickly increases. A specially important increase is noticed in the fat, whose quantity is not very great even on the fourteenth day, but after that it becomes considerable. The quantity of protein bodies and albuminoids soluble in water grows continually and regularly in such a way that their absolute quantity increases, while their relative quantity remains nearly unchanged. LIEBERMANN found no gelatine in the embryo of the hen. The embryo does not contain any gelatine-forming substance until the tenth day, and from the fourteenth day on it contains a body which, when boiled with water, gives a substance similar to chondrin. A body similar to mucin occurs in the embryo when about six days old, but then disappears. The quantity of hæmoglobin shows a continual increase compared with the weight of the body. LIEBERMANN found that the relationship of the hæmoglobin to the body weight was 1:728 on the eleventh day and 1:421 on the twenty-first day.

By means of BERTHELOT'S thermometric methods TANGL has determined the chemical energy present at the beginning and end of the development of the embryo of the sparrow's and hen's eggs. The difference was considered as work of development. He found that the chemical energy necessary for the development of 1 gram of ripe or nearly ripe hen's embryo (Plymouth egg) was equal to 658 calories. This energy originated chiefly from the fat. Of the total chemical energy utilized, two-thirds was used for the construction of the embryo and one-third transformed into other forms of energy as work of development. Still more recent researches of

BOHR and HASSELBALCH[1] show that none of the transformed chemical energy is used in the construction of the embryo, as it leaves the egg almost entirely as heat.

By their investigations on the development of the trout egg TANGL and FARKAS[2] have found that the loss in weight of each egg which had an average weight of 88 milligrams was 4.9 milligrams during the 42 days of incubation, of which 4.11 milligrams was water and 0.722 milligram dry substance with 0.367 milligram C. The eggs loose no nitrogen and no fat. The fat content increases a little, and indeed, as these authors believe, at the expense of the proteins. The chemical energy used during development was 6.68 gram-calories.

The tissue of the **placenta** has not thus far been the subject of detailed chemical investigation. It contains a protein which coagulates at 60–65° (BOTTAZZI and DELFINO), also glycocoll and a proteolytic as well as a diastatic enzyme (ASCOLI, RAINERI, BERGELL, and LIEPMANN[3]). In the edges of the placenta of bitches and of cats a crystallizable orange-colored pigment (bilirubin?) has been found, and also a green amorphous pigment, whose relationship to biliverdin is not known.[4]

From the cotyledons of the placenta in ruminants a white or faintly rose-colored creamy fluid, the *uterine milk*, can be obtained by pressure. It is alkaline in reaction, but becomes acid quickly. Its specific gravity is 1.033–1.040. It contains as form-elements fat-globules, small granules, and epithelium-cells. There have been found 81.2–120.9 p. m. solids, 61.2–105.6 p. m. protein, about 10 p. m. fat, and 3.7–8.2 p. m. ash in the uterine milk.

The fluid occurring in the so-called GRAPE-MOLE (Mola racemosa) has a low specific gravity, 1.009–1.012, and contains 19.4–26.3 p. m. solids with 9–10 p. m. protein bodies and 6–7 p. m. ash.

The **amniotic fluid** in women is thin, whitish, or pale yellow; sometimes it is somewhat yellowish brown and cloudy. White flakes separate. The form-elements are *mucus-corpuscles*, *epithelium-cells*, *fat-drops*, and *lanugo hair*. The odor is stale, the reaction neutral or faintly alkaline. The specific gravity is 1.002–1.028.

The amniotic fluid contains the constituents of ordinary transudates. The amount of solids at birth is hardly 20 p. m. In the earlier stages of pregnancy the fluid contains more solids, especially proteins. Among the protein bodies WEYL found one substance similar to *vitellin*, and with great probability also *seralbumin*, besides small quantities of *mucin*. Enzymes of various kinds (pepsin, diastase, thrombin, lipase) occur, according to BONDI. *Sugar* is regularly found in the amniotic fluid of cows, but not in human beings. In the ox, pig, and goat GÜRBER and GRÜNBAUM have also found levulose. The human amniotic fluid also contains some *urea*, *uric*

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[1] Tangl, Pflüger's Arch., **93**; Bohr and Hasselbalch, Skand. Arch. f. Physiol., **14**.

[2] Pflüger's Arch., **104**.

[3] Bottazzi and Delfino, Centralbl. f. Physiol., **18**, 114; Ascoli, *ibid.*, **16**; Raineri, Biochem. Centralbl., **4**, 428; Bergell and Liepmann, Münch. med. Wochenschr., 1905.

[4] See Etti, Maly's Jahresber., **2**, 287, and Preyer, Die Blutkristalle, Jena, 1871.

acid, and *allantoin*. The quantity of these may be increased in hydramnion (PROCHOWNICK, HARNACK), which depends on an increased secretion by the kidneys and skin of the fœtus. Creatine and lactates are doubtful constituents of the amniotic fluid. The quantity of urea in the amniotic fluid is, according to PROCHOWNICK, 0.16 p. m. In the fluid in hydramnion PROCHOWNICK and HARNACK found respectively 0.34 and 0.48 p. m. urea. The chief mass of the solids consists of salts. The quantity of chlorides (NaCl) is 5.7–6.6 p. m. The molecular concentration of the amniotic fluid is somewhat lower than that of the blood, which is no doubt due to a dilution by the fœtal urine (ZANGEMEISTER and MEISSL [1]).

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[1] Weyl, Arch. f. (Anat. u.) Physiol., 1876; Bondi, Centralbl. f. Gynäkol., 1903; Prochownick, Arch. f. Gynäk., 11, also Maly's Jahresber., 7, 155; Harnack, Berlin. klin. Wochenschr., 1888 No. 41; Zangemeister and Meissl, Münch. med. Wochenschr., 1903; Gürber and Grünbaum, *ibid.*, 1904.

CHAPTER XIV.

MILK.

THE chemical constituents of the *mammary glands* have been little studied. The cells are rich in protein and *nucleoproteids.* Among the latter we have one that yields pentose and guanine, but no other purine base, on boiling with dilute mineral acids. This *compound proteid,* investigated by ODENIUS, contains as an average the following: 17.28 per cent N, 0.89 per cent S, and 0.277 per cent P. Besides this proteid we have at least one other, as MANDEL and LEVENE and LOEBISCH[1] have isolated a nucleic acid from the mammary gland, which, like the thymonucleic acids, yielded adenine, guanine, thymine, and cytosine. This nucleic acid also gave the pentose reactions and yielded abundance of levulinic acid. Besides this nucleic acid, MANDEL and LEVENE[2] isolated from the glands a glucothionic acid with 2.65 per cent S and 4.38 per cent N. We cannot state what relation these substances bear to that constituent of the gland found by BERT, which on boiling with dilute mineral acids yielded a reducing substance. A similar substance, which acts perhaps as a step towards the formation of lactose, has also been observed by THIERFELDER. It is to be expected that these bodies are steps in the formation of milk-sugar; still we have no point of support for such an assumption, and the recent investigations seem to indicate that the milk-sugar is produced in the glands by a transformation of the sugar of the blood. *Fat* seems, at least in the secreting glands, to be a never-failing constituent of the cells, and this fat may be observed in the protoplasm as large or small globules similar to milk-globules. The extractive bodies of the mammary glands have been little investigated, but among them are found considerable amounts of *purine bases.* The mammary glands contain also a proteolytic enzyme which, according to HILDEBRANDT,[3] occurs to a much greater extent in the active gland as compared with the inactive one.

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[1] Odenius, Maly's Jahresber., **30**; Mandel and Levene, Zeitschr. f. physiol. Chem., **46**; Loebisch, Hofmeister's Beiträge, 8.

[2] Zeitschr. f. physiol. Chem., **45**.

[3] Bert, Compt. rend., **98**; Thierfelder, Pflüger's Arch., **34**, and Maly's Jahresber. **13**; Hildebrandt, Hofmeister's Beiträge, **5**.

As human milk and the milk of animals are essentially of the same constitution, it seems best to speak first of the one most thoroughly investigated, namely, cow's milk, and then of the essential properties of the remaining important kinds of milk.[1]

Cow's Milk.

Cow's milk, like every other kind, forms an emulsion which consists of very finely divided fat suspended in a solution consisting chiefly of protein bodies, milk-sugar, and salts. Milk is non-transparent, white, whitish yellow, or in thin layers somewhat bluish white, of a faint, insipid odor and mild, faintly sweetish taste. The specific gravity is 1.028 to 1.0345 at 15° C. The freezing-point is 0.54–0.59° C., average 0.563° C., and the molecular concentration 0.298.

The reaction of perfectly fresh milk is generally amphoteric towards litmus. The extent of the acid and alkaline part of this amphoteric reaction has been determined by different investigators, especially THÖRNER, SEBELIEN, and COURANT.[2] The results differ somewhat with the indicators used, and moreover the milk from different animals, as well as that from the same animal at different times during the lactation period, varies somewhat. COURANT has determined the alkaline part by N/10 sulphuric acid, using blue lacmoid as indicator, and the acid part by N/10 caustic soda, using phenolphthalein as indicator. He found, as an average for the first and last portions of the milking of twenty cows, that 100 c.c. milk had the same alkaline reaction toward blue lacmoid as 41 c.c. N/10 caustic soda, and the same acid reaction toward phenolphthalein as 19.5 c.c. N/10 sulphuric acid. The actual reaction of cow's milk, which follows from the electrometric estimation, is, on the contrary, according to FOA,[3] nearly neutral, like the reaction of animal fluids and tissues in general.

Milk gradually changes when exposed to the air, and its reaction becomes more and more acid. This depends on a gradual transformation of the milk-sugar into lactic acid, caused by micro-organisms.

Perfectly fresh amphoteric milk does not coagulate on boiling, but forms a pellicle consisting of coagulated casein and lime-salts, which rapidly reforms after being removed. Even after passing a current of carbon dioxide through the fresh milk it does not coagulate on boiling. In proportion as the formation of lactic acid advances this behavior changes, and soon a stage is reached when the milk, which has previously had carbon dioxide passed through it, coagulates on boiling. At a second stage it coagulates alone on heating; then it coagulates by passing carbon dioxide alone without boiling; and lastly, when the formation of lactic acid is sufficient, it coagulates spontaneously at the ordinary temperature, forming a solid mass. It may also happen, especially in the warmth, that the casein-clot contracts and a yellowish or yellowish-green acid liquid (acid whey) separates.

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[1] A very complete reference to the literature on milk may be found in Raudnitz's "Die Bestandteile der Milch," in Ergebnisse der Physiol., 2, Abt. 1. The literature of the last few years may be found in the references by Raudnitz, Monatsschrift f. Kinderheilkunde.

[2] Thörner, Maly's Jahresber., 22; Sebelien, *ibid.*; Courant, Pflüger's Arch., **50**.

[3] Compt. rend. Soc. biolog. (58), 59, 51.

Milk may undergo various fermentations. Lactic-acid fermentation, brought about by HÜPPE's lactic-acid bacillus and also other varieties, takes first place. In the spontaneous souring of milk we generally consider the formation of lactic acid as the most essential product, but a formation of succinic acid may also take place, and in certain bacterial decompositions of milk, succinic acid and no lactic acid is formed. The materials from which these two acids are formed are lactose and lactophosphocarnic acid. Besides the lactic acids, the optically inactive as well as the dextro and levo acids, and succinic acid, volatile fatty acids, such as acetic acid, butyric acid, and others, may be formed in the bacterial decomposition of milk.

Milk sometimes undergoes a peculiar kind of coagulation, being converted into a thick, ropy, slimy mass (thick milk). This conversion depends upon a peculiar change in which the milk-sugar is made to undergo a slimy transformation. This transformation is caused by special micro-organisms.

If the milk is sterilized by heating and contact with micro-organisms prevented, the formation of lactic acid may be entirely stopped. The production of acid may also be prevented, at least for some time, by many antiseptics, such as salicylic acid, thymol, boric acid, and other bodies.

If freshly drawn amphoteric milk is treated with rennet, it coagulates quickly, especially at the temperature of the body, to a solid mass (curd) from which a yellowish fluid (sweet whey) is gradually pressed out. This coagulation occurs without any change in the reaction of the milk, and therefore it is distinct from the acid coagulation.

In cow's milk we find as form-elements a few colostrum corpuscles (see Colostrum) and a few pale nucleated cells. The number of these form-elements is very small compared with the immense amount of the most essential form-constituents, the milk-globules.

The Milk-globules. These consist of extremely small drops of fat whose number is, according to WOLL,[1] 1.06–5.75 millions in 1 c.mm., and whose diameter is 0.0024–0.0046 mm. and 0.0037 mm. as an average for different kinds of animals. It is unquestionable that the milk-globules contain fat, and we consider it as positive that all the milk-fat exists in them. Another disputed question is whether the milk-globules consist entirely of fat or whether they also contain protein.

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[1] On the Conditions Influencing the Number and Size of Fat-globules in Cow's Milk, Wisconsin Exp. Station, 6, 1892.

According to the observations of Ascherson,[1] drops of fat, when dropped in an alkaline protein solution, are covered with a fine albuminous coat, a so-called *haptogen-membrane*. As milk on shaking with ether does not give up its fat, or only very slowly in the presence of a great excess of ether, and as this takes place very readily after the addition of acids or alkalies, which dissolve proteins, it was formerly thought that the fat-globules of the milk were enveloped in a protein coat. A true membrane has not been detected; and since, when no means of dissolving the protein is resorted to—for example, when the milk is precipitated by carbon dioxide after the addition of very little acetic acid, or when it is coagulated by rennet—the fat can be very easily extracted by ether, the theory of a special albuminous membrane for the fat-globule has been generally abandoned. The observations of Quincke[2] on the behavior of the fat-globules in an emulsion prepared with gum have led, at the present time, to the conclusion that each fat-globule in the milk is surrounded by a stratum of casein solution held by molecular attraction, and this prevents the globules from uniting with each other. Everything that changes the physical condition of the casein in the milk or precipitates it must necessarily help the solution of the fat in ether, and it is in this way that the alkalies, acids, and rennet act.

V. Storch has shown, in opposition to these views, that the milk-globules are surrounded by a membrane of a special slimy substance. This substance is very insoluble, contains 14.2–14.79 per cent nitrogen, and yields a sugar, or at least a reducing substance, on boiling with hydrochloric acid. It is neither casein nor lactalbumin, but seems to all appearances to be identical with the so-called "stroma substance" detected by Radenhausen and Danilewsky. Storch was able to show, by staining the fat-globules with certain dyes, that this substance enveloped them like a membrane. Recently Völtz has given further proofs of the view that the fat-globules probably have a membrane, which according to him is a very labile formation of variable composition. Droop-Richmond and Bonnema,[3] on the other hand, present several reasons in opposition to Storch's view. If Storch's observation that the purified fat-globules contain a special protein substance differing from the dissolved proteins of the milk is correct, then the assumption as to a special body forming a membrane or stroma of the fat-globules becomes very probable.

The milk-fat which is obtained under the name of butter consists chiefly of *olein* and *palmitin*. Besides these it contains, as triglycerides,

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[1] Arch. f. Anat. u. Physiol., 1840.

[2] Pflüger's Arch., **19**.

[3] V. Storch, see Maly's Jahresber., **27**; Radenhausen and Danilewsky, Forschungen auf dem Gebiete der Viehhaltung (Bremen, 1880), Heft 9; Völtz, Pflüger's Arch., **102**; Droop-Richmond, see Chem. Centralbl., 1904, 2, 356; Bonnema, *ibid.*, 1243.

myristic acid, *stearic acid*, small amounts of *lauric acid*, *arachidic acid*, and *dioxystearic acid*, besides *butyric acid* and *caproic acid*, traces of *caprylic acid* and *capric acid*. It must not be accepted that triglycerides of volatile fatty acids occur, but rather mixed triglycerides of volatile and non-volatile fatty acids (RIEGEL). Milk-fat also contains a small quantity of *lecithin* and *cholesterin* and a yellow *coloring-matter*. The quantity of volatile fatty acids in butter is, according to DUCLAUX, on an average about 70 p. m., of which 37–51 p. m. is butyric acid and 30–33 p. m. is caproic acid. The non-volatile fat consists of $\frac{3}{10}$–$\frac{4}{10}$ olein, and the remainder is chiefly palmitin. The composition of butter is not constant, but varies considerably under different circumstances.[1] According to LEMUS[2] the small fat-globules contain more olein and less volatile acids than the large globules.

The *milk-plasma*, or that fluid in which the fat-globules are suspended, contains several different proteins, the statements as to the number and nature of which are somewhat at variance. The three following, *casein*, *lactalbumin*, and *lactoglobulin*, have been closest studied and are well characterized. The milk-plasma contains two carbohydrates, of which the one, *lactose*, is of great importance. It also contains extractive bodies, traces of *urea*, *creatine*, *creatinine*, *orotic acid*, *hypoxanthine* (?), *lecithin*, *cholesterin*, *citric acid* (SOXHLET and HENKEL[3]), and lastly also *mineral bodies* and *gases*.

Casein. This protein substance, which thus far has been detected positively only in milk, belongs to the nucleoalbumins, and differs from the albuminates chiefly by its content of phosphorus and by its behavior with the rennet enzyme. Casein from cow's milk has the following composition: C 53.0, H 7.0, N 15.7, S 0.8, P 0.85, and O 22.65 per cent. Its specific rotation is, according to HOPPE-SEYLER, somewhat variable; in neutral solution it is $(\alpha)_D = -80°$; its faintly alkaline solution has a stronger rotation, namely, -97.8 to $-111.8°$, in a solution of N/10–N/5 NaOH (LONG[4]). The question whether the casein from different kinds of milk is identical or whether there are several different caseins is still disputed.

Casein when dry appears like a fine white powder, which has no measurable solubility in pure water (LAQUEUR and SACKUR). Casein is only very slightly soluble in the ordinary neutral-salt solutions. According to

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[1] Riegel, Maly's Jahresber., **34**; Duclaux, Compt. rend., **104**. Various statements as to the composition of milk-fat can be found in Koefoed, Bull.d. l' Acad. Roy. Danoise, 1891, and Wanklyn, Chemical News, **63**; Browne, Chem. Centralbl., 1899, **2**, 883.

[2] See Maly's Jahresber., **34**.

[3] Cited from F. Söldner, Die Salze der Milch, etc., Landwirthsch. Versuchsstation, **35**, Separatabzug, 18.

[4] Hoppe-Seyler, Handb. d. physiol. u. pathol. chem. Analyse, **7**. Aufl., 368; Long, Journ. Amer. Chem. Soc. **27**.

Arthus it dissolves rather easily in a 1 per cent solution of sodium fluoride, ammonium or potassium oxalate. It is at least a tetrabasic acid, whose equivalent weight is 1135 according to **Laqueur** and **Sackur**,[1] and whose molecular weight is four or six times this. The salts are split hydrolytically. It dissolves readily in water with the aid of alkali or alkaline earths, also calcium carbonate, from which it expels carbon dioxide. If casein is dissolved in lime-water and this solution carefully treated with very dilute phosphoric acid until it is neutral in reaction, the casein appears to remain in solution, but is probably only swollen as in milk, and the liquid contains at the same time a large quantity of calcium phosphate without any precipitate or any suspended particles being visible. The casein solutions containing lime are opalescent and have on warming the appearance of milk deficient in fat (which is also true for the salts of casein with the alkaline earths). Therefore it is not impossible that the white color of the milk is due partly to the casein and calcium phosphate. **Söldner** has prepared two calcium compounds of casein with 1.55 and 2.36 per cent CaO, and these compounds are designated di- and tricalcium casein by **Courant**.[2]

According to **Laqueur**,[3] who has determined the electrical conductivity and the internal friction of casein solutions, all casein-salt solutions consist of a mixture of casein ions (with different amounts of H which can be split off electrolytically) and unsplit casein (produced by hydrolysis). By the gradual addition of alkali to the casein he found no sharp distinguishing point and therefore proposes to drop the names mono-, di-, and tricasein.

Casein solutions do not coagulate on boiling, but solutions of casein-lime are covered, like milk, with a pellicle. They are precipitated by very little acid, but the presence of neutral salts retards the precipitation. A casein solution containing salt or ordinary milk requires, therefore, more acid for precipitation than a salt-free solution of casein of the same concentration. The precipitated casein dissolves very easily again in a small excess of hydrochloric acid, but less easily in an excess of acetic acid. The combination between casein and acid, and especially the combination with lactic acid, which has been carefully studied by **Laxa**,[4] are, like other protein and acid compounds, precipitated by neutral salts. These acid solutions are precipitated by mineral acids in excess. Casein is precipitated from neutral solutions or from milk by common salt containing calcium or magnesium sulphate in substance, without changing its properties. Metallic salts, such as alum, zinc sulphate, and copper sulphate, completely precipitate the casein from neutral solutions.

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[1] Laqueur and Sackur, Hofmeister's Beiträge, 3; M. Arthus, Thèses presentées à la faculté des sciences de Paris, 1893.

[2] Söldner, Die Salze der Milch, etc.; Courant, l. c. In regard to the salts of casein see the investigations of Söldner, Maly's Jahresber., 25, and J. Röhmann, Berlin. klin. Wochenschr., 1895. See also Raudnitz, Ergebnisse der Physiol., 2, Abt. 1.

[3] Hofmeister's Beiträge, 7.

[4] Milchwirtsch. Centralbl., 1905, Heft 12.

On drying at 100° C., casein, according to LAQUEUR and SACKUR, decomposes and splits into two bodies. One of these, called *caseid*, is insoluble in dilute alkalies, while the other, the *isocasein*, is soluble therein. The isocasein is a stronger acid and has other precipitation limits and a somewhat lower equivalent weight than the casein.

The property which is the most characteristic of casein is that it coagulates with rennet in the presence of a sufficiently great amount of lime-salts. In solutions free from lime-salts the casein does not coagulate with rennet, but it is changed so that the solution (even if the enzyme is destroyed by heating) yields a coagulated mass, having the properties of a curd, if lime-salts are added. The rennet enzyme, rennin, has therefore an action on casein even in the absence of lime-salts. These last are only necessary for the coagulation or the separation of the curd, and the process of coagulation is hence a two-phase process. The first phase is the transformation of the casein by the rennin, the second is the visible coagulation caused by the lime-salts. This fact, which was first proved by HAMMARSTEN, was later confirmed by ARTHUS and PAGÈS and recently closely studied by FULD, SPIRO, and LAQUEUR.[1]

The curd formed on the coagulation of milk contains large quantities of calcium phosphate. According to SOXHLET and SÖLDNER, the soluble lime-salts are of essential importance only in coagulation, while the calcium phosphate is without importance. According to COURANT, the calcium-casein on coagulation may carry down with it, if the solution contains dicalcium phosphate, a part of this as tricalcium phosphate, leaving monocalcium phosphate in the solution. A solution of calcium-casein is not coagulated by rennin alone but only when soluble lime-salts are added. Milk or casein solutions may indeed be precipitated without rennin by the addition of a sufficiently large amount of calcium chloride. We are not quite clear as to the importance of the lime-salts for the rennin coagulation, and the views are still somewhat variable on this question. The same is true for the chemical processes going on in rennin coagulation. If one makes use of a pure solution of casein and as pure rennin as possible, then after coagulation it is always found that the filtrate contains very small amounts of a proteid, the *whey-proteid*, which has other properties and a lower content of nitrogen (13.2 per cent N, KÖSTER[1]) than the casein. The chief portion of the casein, sometimes given as more than 90 per cent, separates on coagulation as a body, the *paracasein* (or curd), which is closely related to casein. The question whether a cleavage of the casein takes place here is still unsettled. The paracasein[2] is not further changed by the rennet enzyme; it is much more readily precipitated by $CaCl_2$ than a casein solution of the same concentration, and the precipitation limits for saturated ammonium-sulphate solution, the upper as well as the lower limit, lie, according to LAQUEUR, lower with paracasein than with casein. The internal friction of paracasein solutions is also, according to him, less than that of the casein solutions and indeed even to 20 per cent.

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[1] See Maly's Jahresber., 2 and 4; also Hammarsten, Zur Kenntnis des Kaseïns und der Wirkung des Labfermentes, Nova Acta Reg. Soc. Scient. Upsala, 1877, Festschrift; Zeitschr. f. physiol. Chem., 22; Arthus et Pagès, Arch. de Physiol. (5), 2, and Mém. Soc. biol., 43; Fuld, Hofmeister's Beiträge, 2, and Ergebnisse der Physiol., 1, Abt. 1, where a good review of the literature may be found; Spiro, Hofmeister's Beiträge, 6 and 7, with Reichel, *ibid.*, 7 and 8; Laqueur, *ibid.*, 7.

In the processes of rennet coagulation we may, as REICHEL and SPIRO have shown, have a diminution of the action by an apparent consumption of the ferment. This diminution is indeed, as the above investigators found, not caused by the process of rennet coagulation and is therefore not to be considered as a consumption of the enzyme. It depends upon a division of the rennin between the curd and the whey taking place according to a constant factor.

Fresh, unchanged milk does not, as is known, coagulate on boiling; but in not too rapid action of rennin a state may be observed in which the milk coagulates on heating (metacasein reaction). A solution of paracasein lactate, according to LAXA, coagulates with rennin the same as a solution of casein lactate, which indicates, according to LAXA, that the paracasein is transformed into casein again by the lactic acid. But as a precipitation of the paracasein from the acid solution is perhaps a pepsin action, the transformation of the paracasein into casein by the lactic acid must not be considered as proved. As the commercial rennet extracts may contain also other enzymes besides rennin, the formation of proteoses in rennin coagulation, as observed by E. PETRY,[3] must not be considered as a rennin action without further study.

In the digestion of casein with pepsin-hydrochloric acid primarily a phosphorized proteose is formed, from which then the pseudonuclein is split off (SALKOWSKI). The quantity thus split off is very variable, as shown by the researches of SALKOWSKI, HAHN, MORACZEWSKI, SEBELIEN, and ZAITSCHEK.[4] The amount of phosphorus in the pseudonucleins obtained also varies considerably. According to SALKOWSKI the quantity of pseudonuclein split off is dependent upon the relationship between the casein and the digestion fluid, e.g., the quantity of the pseudonucleins diminishes as the pepsin-hydrochloric acid increases. In the presence of 500 grams of pepsin-hydrochloric acid to 1 gram of casein SALKOWSKI digested the latter completely without obtaining any pseudonuclein.

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[1] See Maly's Jahresber., **11**.

[2] It has been recently proposed to designate the ordinary casein as caseinogen and the curd as casein. Although such a proposition is theoretically correct, it leads in practice to confusion. On this account the author calls the curd paracasein, according to Schulze and Röse (Landwirthsch. Versuchsstat., **31**). A summary of the literature on the casein coagulation may be found in E. Fuld, Ergebnisse der Physiol., **1**; Raudnitz, *ibid.*, **2**; and Laqueur, Biochem. Centralbl., 4, 344.

[3] Laxa, l. c.; Petry, Wien. klin. Wochenschr., 1906.

[4] Salkowski, Zeitschr. f. physiol. Chem., **27**; Salkowski and Hahn, Pflüger's Arch., **59**; Salkowski, *ibid.*, **63**; v. Moraczewski, Zeitschr. f. physiol. Chem., **20**; Sebelien *ibid.*, **20**; Zaitschek, Pflüger's Arch., **104**.

In peptic as well as tryptic digestion a part of the organic phosphorus is split off as orthophosphoric acid, the quantity increasing as the digestion progresses. Another part of the phosphorus is retained in organic combination in the proteoses as well as in the true peptones (SALKOWSKI, BIFFI, ALEXANDER[1]).

From the products of peptic digestion of casein, after the separation of the pseudonuclein, SALKOWSKI[2] has isolated an acid rich in phosphorus. He considers this a *paranucleic acid*. It is soluble in water, insoluble in alcohol, levorotatory, and has the following composition: C 42.51–42.96, H 6.97–7.09, N 13.25–13.55, and P 4.05–4.31 per cent. The acid differs from the nucleic acids in that it gives the biuret test and a faint xanthoproteic reaction. Presupposing its purity, it is not an acid comparable to the nucleic acids.

Casein may be prepared in the following way: The milk is diluted with 4 vols. of water and the mixture treated with acetic acid to 0.75—1 p. m. Casein thus obtained is purified by repeatedly dissolving in water with the aid of the smallest quantity of alkali possible, by filtering and reprecipitating with acetic acid and thoroughly washing with water. Most of the milk-fat is retained by the filter on the first filtration, and the casein contaminated with traces of fat is purified by treating with alcohol and ether.

Lactoglobulin was obtained by SEBELIEN from cow's milk by saturating it with NaCl in substance (which precipitated the casein) and saturating the filtrate with magnesium sulphate. As far as it has been investigated it had the properties of serglobulin; the globulin isolated by TIEMANN[3] from colostrum had nevertheless a markedly low content of carbon, namely, 49.83 per cent.

Lactalbumin was first prepared in a pure state from milk by SEBELIEN. Its composition is, according to him, C 52.19, H 7.18, N 15.77, S 1.73, O 23.13 per cent. Lactalbumin has the properties of the albumins, and it crystallizes according to WICHMANN[4] in forms similar to ser- or ovalbumin. It coagulates, according to the concentration and the amount of salt in solution, at 72–84° C. It is similar to seralbumin, but differs from it in having a considerably lower specific rotatory power: $(\alpha)_D = -37°$.

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[1] Salkowski, l. c.; Biffi, Virchow's Arch., **152**; Alexander, Zeitschr. f. physiol. Chem., **25**.

[2] Zeitschr. f. physiol. Chem., **32**.

[3] *Ibid.*, **25**.

[4] Sebelien, Zeitschr. f. physiol. Chem., **9**; Wichmann, *ibid.*, **27**.

The principle of the preparation of lactalbumin is the same as for the preparation of seralbumin from serum. The casein and the globulin are removed by $MgSO_4$ in substance and the filtrate treated as previously stated (page 182).

The occurrence of other proteins, such as *proteoses* and *peptones*, in milk has not been positively proved. These bodies are easily produced as laboratory products from the other proteins of the milk. Such a laboratory product is MILLON'S and COMAILLE'S *lactoprotein*, which is a mixture of a little casein with changed albumin, and proteose [1] which is formed by chemical action. In regard to *opalisin*, see Human Milk, p. 531.

Milk also contains, according to SIEGFRIED,[2] a *nucleon* related to phosphocarnic acid, and which yields fermentation lactic acid (instead of paralactic acid) and a special carnic acid, *orylic acid* (instead of muscle carnic acid), as cleavage products. Lactophosphocarnic acid may be precipitated as an iron compound from the milk freed from casein and coagulable proteins as well as from earthy phosphates.

Milk also contains *enzymes* of various kinds. Of these we must mention *catalase*, *oxidases*, *peroxidases*, and *reductases*, but the statements as to their occurrence in the milk from different animals are not unanimous. An *amylolytic enzyme* which converts starch into maltose occurs especially in human milk, while it is absent in cow's milk or occurs only to a slight extent. A *fermentation enzyme* which in the absence of micro-organisms decomposes the lactose into lactic acid, alcohol, and CO_2, occurs, according to STOKLASA [3] and his co-workers, in cow's milk as well as in human milk. Human milk, as well as cow's milk, contains a *lipase* which has the property at least of acting upon monobutyrin. BABCOCK and RUSSEL have found in these two kinds of milk, as well as certain others, a proteolytic enzyme which they call *galactase* and which is allied to trypsin, but differs therefrom in that it develops ammonia from milk even in the early stages of digestion. The occurrence of such an enzyme is denied by ZAITSCHEK and v. SZONTAGH, but on the other hand VANDEVELDE, DE WAELE, and SUGG [4] confirm the occurrence of a proteolytic enzyme in milk.

Orotic acid, $C_5H_{11}N_2O_4.2H_2O$, is the name given by BISCARO and BELLONI [5] to a new constituent of milk which they have discovered. This acid, which can be precipitated by basic lead acetate from whey free from protein, is slightly soluble in water, crystalline, and gives several crystalline salts. The monomethyl and ethyl esters of this acid are also known. It yields urea on treatment with potassium permanganate.

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[1] See Hammarsten, Maly's Jahresber., 6, 13.

[2] Zeitschr. f. physiol. Chem., 21 and 22.

[3] See Chem. Centralbl., 1905, 1, 107.

[4] Babcock and Russel, Centralbl. f. Bakt. u. Parisitenkunde (II), 6, and Maly's Jahresber., 31; Zaitschek and v Szontagh, Pflüger's Arch., 104; Vandevelde, de Waele, and Sugg, Hofmeister's Beiträge, 5.

[5] See Chem. Centralbl., 1905, 2, 63.

Lactose, MILK-SUGAR, $C_{12}H_{22}O_{11}+H_2O$. This sugar, on hydrolysis, can be split into two hexoses, *dextrose* and *galactose*. It yields mucic acid, besides other organic acids, by the action of dilute nitric acid. Levulinic acid is formed, besides formic acid and humin substances, by the stronger action of acids. By the action of alkalies, amongst other products we find lactic acid and pyrocatechin.

Milk-sugar occurs, as a rule, only in milk, but it has also been found in the urine of pregnant women on stagnation of milk, as well as in the urine after partaking of large quantities of the same sugar.

Lactose, of which, according to TANRET,[1] there are three modifications, occurs ordinarily as colorless rhombic crystals with 1 molecule of water of crystallization, which is driven off by slowly heating to 100° C., but more easily at 130–140° C. At 170° to 180° C. it is converted into a brown amorphous mass, lactocaramel, $C_6H_{10}O_5$. On quickly boiling down a milk-sugar solution, anhydrous milk-sugar separates out. Milk-sugar dissolves in 6 parts cold or in 2.5 parts boiling water; it has a faintly sweetish taste. It does not dissolve in ether or absolute alcohol. Its solutions are dextrogyrate. The rotatory power, which on heating the solution to 100° C. becomes constant, is $(\alpha)_D=+52.5°$. Milk-sugar combines with bases; the alkali combinations are insoluble in alcohol.

Milk-sugar is not fermentable with pure yeast. It undergoes, on the contrary, alcoholic fermentation by the action of certain schizomycetes, and according to E. FISCHER[2] the milk-sugar is first split into dextrose and galactose by an enzyme, *lactase,* existing in the fungus. The preparation of milk-wine, "*kumyss*," from mare's milk and "*kephir*" from cow's milk is based upon this fact. Other micro-organisms also take part in this change, causing a lactic-acid fermentation of the milk-sugar.

Lactose responds to the reactions of dextrose, such as MOORE'S, TROMMER'S, and RUBNER'S, and the bismuth test. It also reduces mercuric oxide in alkaline solutions. After warming with phenylhydrazine acetate it gives on cooling a yellow crystalline precipitate of phenyllactosazone, $C_{24}H_{32}N_4O_9$. It differs from cane-sugar by giving positive reactions with MOORE'S or TROMMER'S and the bismuth test, and also in that it does not darken when heated with anhydrous oxalic acid to 100° C. It differs from dextrose and maltose by its solubility and crystalline form, but especially by its not fermenting with yeast and by yielding mucic acid with nitric acid.

The osazone obtained with phenylhydrazine acetate, which melts at 200° C., differs from the other osazones by being inactive when 0.2 gram is dissolved in 4 c.c. of pyridine and 6 c.c. of absolute alcohol and viewed through a layer 10 centimetres long (NEUBERG[3]).

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[1] Bull. Soc. chim. (3), **13**. [2] Ber. d. d. chem. Gesellsch., **27**. [3] *Ibid.*, **32**.

For the preparation of milk-sugar we make use of the by-product in the preparation of cheese, the sweet whey. The protein is removed by coagulation with heat, and the filtrate evaporated to a syrup. The crystals which separate after a certain time are recrystallized from water after decolorizing with animal charcoal. A pure preparation may be obtained from the commercial milk-sugar by repeated recrystallization. The quantitative estimation of milk-sugar may be performed either by the polaristrobometer or by means of titration with FEHLING'S solution. Ten c.c. of FEHLING'S solution correspond to 0.0676 gram of milk-sugar in 0.5–1.5 per cent solution after boiling for six minutes. (In regard to FEHLING'S solution and the titration of sugar see Chapter XV.)

RITTHAUSEN has found another carbohydrate in milk which is soluble in water, non-crystallizable, which has a faint reducing action, and which yields on boiling with an acid a body having a greater reducing power. LANDWEHR considers this as animal gum, and BECHAMP[1] as dextrin.

The *mineral bodies* of milk will be treated in connection with its quantitative composition.

The methods for the quantitative analysis of milk are very numerous, and as they cannot all be treated here, we will give the chief points of a few of the methods considered most trustworthy and most frequently employed.

In determining the *solids* a carefully weighed quantity of milk is mixed with an equal weight of heated quartz sand, fine glass powder, or asbestos. The evaporation is first done on the water-bath and finished in a current of carbon dioxide or hydrogen not above 100° C.

The *mineral bodies* are determined by incinerating the milk, using the precautions mentioned in the text-books. The results obtained for the phosphoric acid are incorrect on account of the burning of phosphorized bodies, such as casein and lecithin. We must therefore, according to SÖLDNER, subtract in round numbers 25 per cent from the total phosphoric acid found in the milk. The quantity of sulphate in the ash also depends on the combustion of the proteins.

In the determination of the *total amount of proteins* RITTHAUSEN'S method is employed, namely, the precipitation of the milk with copper sulphate according to the modification suggested by MUNK.[2] He precipitates all the proteins by means of cupric hydrate at boiling heat, and determines the nitrogen in the precipitate by means of KJELDAHL'S method. This modification gives more exact results.

The older method of PULS and STENBERG, in which the precipitant is alcohol, is too complicated and not sufficiently reliable. SEBELIEN has suggested a very good method. Three to four grams of milk are diluted with an equal volume of water, a little common-salt solution added, and the proteins precipitated with an excess of tannic acid. The precipitate is washed with cold water, and then the quantity of nitrogen determined by KJELDAHL'S method. The total nitrogen found when multiplied by 6.37 (casein and lactalbumin contain both 15.7 per cent nitrogen) gives the total quantity of proteins. This method, which is readily performed, gives very good results. I. MUNK used this method in the analysis of woman's milk. In this case the quantity of nitrogen found must be multiplied by 6.34. G. SIMON [1] has found that the precipitation with tannic acid, also with phosphotungstic acid, is the simplest and most accurate. The objection to this and other methods in which the proteins are precipitated is that perhaps other bodies (extractives) may be carried down at the same time (CAMERER and SÖLDNER [2]). It is not known to what extent this takes place.

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[1] Ritthausen, Journ. f. prakt. Chem. (N. F.), 15; Landwehr, foot-note 1, p. 67; Béchamp, Bull. Soc. chim. (3) 6.

[2] Ritthausen, l c.; I. Munk, Virchow's Arch., 134.

A part of the nitrogen in the milk exists as extractives, and this nitrogen is calculated as the difference between the total nitrogen and the protein nitrogen. According to MUNK's analyses about $\frac{1}{16}$ of the total nitrogen belongs to the extractives in cow's milk, and $\frac{1}{11}$ in woman's milk. CAMERER and SOLDNER determine the nitrogen in the filtrate from the tannic-acid precipitate by KJELDAHL's method, and also according to HÜFNER's method (hypobromite). In this way they found 18 milligrams of nitrogen according to HÜFNER (urea, etc.) in 100 grams of cow's milk.

To determine the *casein* and *albumin* separately we may make use of the method first suggested by HOPPE-SEYLER and TOLMATSCHEFF,[3] in which the casein is precipitated by magnesium sulphate. According to SEBELIEN the milk is diluted with its own volume of a saturated magnesium-sulphate solution, then saturated with the salt in substance, and the precipitate then filtered and washed with a saturated magnesium-sulphate solution. The nitrogen is determined in the precipitate by KJELDAHL's method, and the quantity of casein (+globulin) determined by multiplying the result by 6.37. The quantity of lactalbumin may be calculated as the difference between the casein and the total proteins found. The lactalbumin may also be precipitated by tannic acid from the filtrate from the casein precipitate containing $MgSO_4$, after diluting with water, the nitrogen determined by KJELDAHL's method and the result multiplied by 6.37.

SCHLOSSMANN [4] suggests an alum solution, which precipitates the casein, in order to separate the casein from the other proteins, the albumin can be precipitated from the filtrate by tannic acid. The nitrogen in the precipitate is determined by the KJELDAHL's method. This method has recently been tested by SIMON and he recommends it highly.

The *fat* is gravimetrically determined by thoroughly extracting the dried milk with ether, evaporating the ether from the extract, and weighing the residue. The fat may be determined by aerometric means by adding alkali to the milk, shaking with ether, and determining the specific gravity of the fat solution by means of SOXHLET's apparatus. In determining the amount of fat in a large number of samples the lactocrit of DE LAVAL may be used with success. The milk is first mixed with an equal volume of a mixture of glacial acetic and concentrated sulphuric acid, warmed 7–8 minutes on the water-bath, and the mixture poured in graduated tubes, which are placed in the centrifugal machine at 50° C. The height of the layer of fat gives its quantity. The numerous and very exact analyses of **Nilson**[1] have shown that with milks containing small quantities of fat, below 1.5 per cent, the older corrections are unnecessary, and that this method gives excellent results if we use lactic acid treated with 5 per cent hydrochloric acid instead of the above mixture of glacial acetic acid and sulphuric acid. There are numerous other methods for estimating milk-fat but they cannot be considered here.

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[1] Puls, Pflüger's Arch., **13**; Stenberg, Maly's Jahresber., **7**; Sebelien, Zeitschr. f. physiol. Chem., **13**; Simon, *ibid.*, **33**.

[2] Zeitschr. f. Biologie, **33** and **36**.

[3] Hoppe-Seyler, Med. chem. Untersuch., 272.

[4] Zeitschr. f. physiol. Chem., **22**.

In determining the *milk-sugar* the proteins are first removed. For this purpose we precipitate either with alcohol, which must be evaporated from the filtrate, or by diluting with water, and removing the casein by the addition of a little acid, and the lactalbumin by coagulation at boiling heat. The sugar is determined by titration with **Fehling's** or **Knapp's** solution (see Chapter XV). The principle of the titration is the same as for the titration of sugar in the urine: 10 c.c. of **Fehling's** solution correspond to 0.0676 gram of milk-sugar; 10 c.c. of **Knapp's** solution correspond to 0.0311–0.0310 gram of milk-sugar, when the saccharine liquid contains about ½–1 per cent of sugar. In regard to the *modus operandi* of the titration we must refer the reader to more complete works and to Chapter XV.

Instead of these volumetric determinations other methods of estimation, such as **Allihn's** method, the polariscope method, and others, may be used. In calculating the analysis or in determining the solids it is of importance to remember, as suggested by **Camerer** and **Söldner**, that the milk-sugar in the residue is anhydrous. Many other methods for determining the milk-sugar have been suggested and recommended.

The *quantitative composition* of cow's milk is naturally very variable. The average obtained by **König**[2] is as follows in 1000 parts:

<table>
<tr><th>Water.</th><th>Solids.</th><th>Casein.</th><th>Albumin.</th><th>Fats.</th><th>Sugar.</th><th>Salts.</th></tr>
<tr><td>871.7</td><td>128.3</td><td>30.2</td><td>5.3</td><td>36.9</td><td>48.8</td><td>7.1</td></tr>
<tr><td></td><td></td><td colspan="2">35.5</td><td></td><td></td><td></td></tr>
</table>

The quantity of *mineral bodies* in 1000 parts of cow's milk is, according to the analyses of **Söldner**, as follows: K_2O 1.72, Na_2O 0.51, CaO 1.98, MgO 0.20, P_2O_5 1.82 (after correction for the pseudonuclein), Cl 0.98 grams. **Bunge**[3] found 0.0035 gram Fe_2O_3. According to **Söldner** the K, Na, and Cl are found in the same quantities in whole milk as in milk-serum. Of the total phosphoric acid 36–56 per cent and of the lime 53–72 per cent is not in simple solution. A part of this lime is combined with the casein; the remainder is found united with the phosphoric acid as a mixture of dicalcium and tricalcium phosphates which is kept dissolved or suspended by the casein. The bases are in excess of the mineral acids in the milk-serum. The excess of the first is combined with organic acids, which correspond to 2.5 p. m. citric acid (**Söldner**).

The *gases* of the milk consist chiefly of CO_2, besides a little N and traces of O. PFLÜGER[1] found 10 vols. per cent CO_2 and 0.6 vol. per cent N calculated at 0° C. and 760 mm. pressure.

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[1] See Maly's Jahresber., **21**.

[2] Chemie der menschlichen Nahrungs- und Genussmittel, 4. Aufl.

[3] Zeitschr. f. Biologie, **10**.

The variation in the composition of cow's milk depends on several circumstances.

The **colostrum,** or the milk which is secreted before calving and in the first few days after, is yellowish, sometimes alkaline, but often acid, of higher specific gravity, 1.046–1.080, and richer in solids than ordinary milk. The colostrum contains, besides fat-globules, an abundance of colostrum-corpuscles—nucleated granular cells 0.005–0.025 mm. in diameter with abundant fat-granules and fat-globules. The fat of colostrum has a somewhat higher melting-point and is poorer in volatile fatty acids than the fat from ordinary milk (NILSON[2]). The iodine equivalent of the colostrum-fat is higher than that of milk-fat. The quantity of cholesterin and lecithin is generally greater. The most apparent difference between it and ordinary milk is that colostrum coagulates on heating to boiling because of the absolutely and relatively greater quantities of globulin and albumin that it contains.[3] The composition of colostrum is very variable. KÖNIG gives as average the following figures in 1000 parts:

Water.	Solids.	Casein.	Albumin and Globulin.	Fat.	Sugar.	Salts.
746.7	253.3	40.4	136.0	35.9	26.7	15.6

The influence which food exercises upon the composition of milk will be discussed in connection with the chemistry of the milk secretion.

In the following table is given the average composition of skimmed milk and certain other preparations of milk:

	Water.	Proteins.	Fat.	Sugar.	Lactic Acid.	Salts.
Skimmed milk......	906.6	31.1	7.4	47.5	...	7.4
Cream.............	655.1	36.1	267.5	35.2	...	6.1
Buttermilk.........	902.7	40.6	9.3	37.3	3.4	6.7
Whey..............	932.4	8.5	2.3	47.0	3.3	6.5

KUMYSS and KEPHIR are obtained, as above stated, by the alcoholic and lactic-acid fermentation of the milk-sugar, the former from mare's milk and the latter from cow's milk. Large quantities of carbon dioxide are formed thereby, and besides the protein bodies of the milk are partly converted into proteoses and peptones, which increase the digestibility. The quantity of lactic acid in these preparations may be about 10–20 p. m. The quantity of alcohol varies from 10 to 35 p. m.

Milk of other Animals. GOAT'S milk has a more yellowish color and another, more specific odor than cow's milk. The coagulum obtained by acid or rennet is more solid and is harder than that from cow's milk. SHEEP'S milk is similar to goat's milk, but has a higher specific gravity and contains a greater amount of solids.

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[1] Pflüger's Arch., **2**.

[2] Nilson, l. c.

[3] See Sebelien, Maly's Jahresber., **18**, and Tiemann, Zeitschr. f. physiol. Chem., **25**. See also Simon, *ibid.*, **33**; Winterstein and Strickler, *ibid.*, **47**.

Mare's milk is alkaline and contains a casein which is not precipitated by acids in lumps or solid masses, but, like the casein from woman's milk, in fine flakes. This casein is only incompletely precipitated by rennet, and it is very similar also in other respects to the casein of human milk. According to Beil[1] the casein from mare's and cow's milk is the same, and the different behavior of the two varieties of milk is due to different amounts of salts and to a different relation between the casein and the albumin. This does not agree with the investigations of Zaitschek and v. Szontagh, who find that the casein from mare's milk, like that from human and ass's milk, is digested by pepsin-hydrochloric acid without leaving a residue. The milk of the ass is claimed by older authorities to be similar to human milk, but Schlossmann finds it considerably poorer in fat. The researches of Ellenberger give similar results, and show great similarity between ass's milk and human milk. The average results were 15 p. m. protein with 5.3 p. m. albumin and 9.4 p. m. casein. This latter, like human casein, does not yield any pseudonuclein on pepsin digestion, which agrees well with the above-mentioned investigations of Zaitschek. The quantity of nucleon was about the same as in woman's milk. The quantity of fat was 15 p. m., and the sugar was 50–60 p. m. Reindeer milk is characterized, according to Werenskiold,[2] by being very rich in fat, 144.6–197.3 p. m., and casein, 80.6–86.9 p. m.

The milk of carnivora (the bitch and cat) is acid in reaction and very rich in solids. The composition of the milk of these animals varies with the composition of the food.

To illustrate the composition of the milk of other animals the following figures, the compilation of König, are given. As the milk of each kind of animal may have a variable composition, these figures should only be considered as examples of the composition of milk of various kinds:[3]

Milk of the	Water.	Solids.	Proteins.	Fat.	Sugar.	Salts.
Dog	754.4	245.6	99.1	95.7	31.9	7.3
Cat	816.3	183.7	90.8	33.3	49.1	5.8
Goat	869.1	130.9	36.9	40.9	44.5	8.6
Sheep	835.0	165.0	57.4	61.4	39.6	6.6
Cow	871.7	128.3	35.5	36.9	48.8	7.1
Horse	900.6	99.4	18.9	10.9	66.5	3.1
Ass	900.0	100.0	21.0	13.0	63.0	3.0
Pig	823.7	167.3	60.9	64.4	40.4	10.6
Elephant	678.5	321.5	30.9	195.7	88.4	6.5
Dolphin	486.7	513.3	....	437.6	....	4.6

Human Milk.

Woman's milk is amphoteric in reaction. According to Courant its reaction is relatively more alkaline than cow's milk, but it has nevertheless a lower absolute reaction for alkalinity as well as for acidity. Courant found between the tenth day and the fourteenth month after confinement practically constant results. The alkalinity, as well as the acidity, was a little lower than in childbed. One hundred c.c. of the milk had the same average alkalinity as 10.8 c.c. N/10 caustic soda, and the same acidity as 3.6 c.c. N/10 acid. The relationship between the alkalinity and the acidity in woman's milk was as 3:1, and in cow's milk as 2.1:1. The actual reaction determined electrometrically is, according to FOÀ,[1] still nearly neutral, like the other kinds of milk.

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[1] Studien über die Eiweissstoffe des Kumys und Kefirs, St. Petersburg, 1886 (Ricker).

[2] Zaitschek, l. c.; Schlossmann, Zeitschr. f. physiol. Chem., 22; Ellenberger, Arch. f. (Anat. u.) Physiol., 1899 and 1902; Werenskiold, Maly's Jahresber., 25.

[3] Details in regard to the milk of different animals may be found in Pröscher, Zeitschr f. physiol. Chem., 24; Abderhalden, *ibid.*, 27.

Human milk also contains fewer fat-globules than cow's milk, but they are larger in size. The specific gravity of woman's milk varies between 1.026 and 1.036, generally between 1.028 and 1.034. It is highest in well-fed and lowest in poorly fed women. The freezing-point is lowered on an average 0.589° C., according to WINTER and PARMENTIER[2] constant at 0.55°, and the molecular concentration is 0.318.

The fat of woman's milk has been investigated by RUPPEL. It forms a yellowish-white mass, similar to ordinary butter, having a specific gravity of 0.966 at 15° C. It melts at 34.0° C. and solidifies at 20.2° C. The following fatty acids can be obtained from the fat, namely, butyric, caproic, capric, myristic, palmitic, stearic, and oleic acids. The fat from woman's milk is, according to RUPPEL and LAVES,[3] relatively poor in volatile fatty acids. The non-volatile fatty acids consist of one-half oleic acid, while among the solid fatty acids myristic and palmitic acids are found to a greater extent than stearic acid.

The essential qualitative difference between woman's and cow's milk seems to lie in the proteins or in the more accurately determined *casein*. A number of older and younger investigators[4] claim that the casein from woman's milk has other properties than that from cow's milk. The essential differences are the following: The casein from woman's milk is precipitated with greater difficulty with acids or salts; it does not coagulate uniformly in the milk after the addition of rennet; it may be precipitated by gastric juice, but dissolves completely and easily in an excess of the same; the casein precipitate produced by an acid is more easily soluble in an excess of the acid; and lastly, the clot formed from the casein of woman's milk does not appear in such large and coarse masses as the casein from cow's milk, but is more loose and flocculent. This last-mentioned fact is of great importance, since it explains the generally admitted fact of the easy digestibility of the casein from woman's milk. We are not clear as to this difference between the digestibility of the cow's casein and human casein, as the first seems to be utilized in the intestinal tract of the infant to the same extent as human casein (P. MÜLLER, RUBNER and HEUBNER[5]).

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[1] Compt. rend. Soc. biolog., 58.

[2] See Maly's Jahresber., 34.

[3] Ruppel, Zeitschr. f. Biologie, **31**; Laves, Zeitschr. f. physiol. Chem., **19**.

[4] See Biedert, Untersuchungen über die chemischen Unterschiede der Menschen- und Kuhmilch (Stuttgart), 1884; Langgaard, Virchow's Arch., **65**; Makris, Studien über die Eiweisskörper der Frauen- und Kuhmilch, Inaug.-Diss. Strassburg, 1876.

[5] Müller, Zeitschr. f. Biologie, **39**; Rubner and Heubner, *ibid.*, **37**.

The question as to whether the above-mentioned differences depend on a decided difference in the two caseins or only on an unequal relationship between the casein and the salts in the two kinds of milk, or upon other circumstances, has not been decided as yet. According to **Szontagh** and **Zaitschek** and also **Wróblewsky**, the casein from human milk does not yield any pseudonuclein on peptic digestion, and hence it cannot be a nucleoalbumin. **Wróblewsky** has found the following for the composition of casein from woman's milk: C 52.24, H 7.32, N 14.97, P 0.68, S 1.117 per cent. According to **Kobrak**[1] woman's casein yields some pseudonuclein, and with repeated solution in alkali and precipitation by an acid it becomes more and more like cow's casein. He therefore suggests the possibility that woman's casein is a compound between a nucleoalbumin and a basic protein.

Woman's milk also contains lactalbumin, besides the casein, and a protein substance, very rich in sulphur (4.7 per cent) and relatively poor in carbon, which **Wróblewsky** calls *opalisin*. The statements as to the occurrence of proteoses and peptones are disputed as in many other cases. No positive proof as to the occurrence of proteoses and peptones in fresh milk has been given.

Even after those differences are eliminated which depend on the imperfect analytical methods employed, *the quantitative composition of woman's milk* is variable to such an extent that it is impossible to give any average results. The recent analyses, especially those made on a large number of samples by **Pfeiffer**, **Adriance**, **Camerer** and **Söldner**,[2] have positively shown that woman's milk is essentially poorer in proteins but richer in sugar than cow's milk. The quantity of protein varies between 10–20 p. m., often amounting to only 15–17 p. m. or less, and is dependent upon the length of lactation (see below). The quantity of fat also varies considerably, but ordinarily amounts to 30–40 p. m. The quantity of sugar should not be below 50 p. m., but may rise to even 80 p. m. About 60 p. m. may be considered as an average, but it should be borne in mind that the quantity of sugar is also dependent upon the length of lactation, as it increases with duration. The amount of mineral bodies varies between 2 and 4 p. m.

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[1] Szontagh, Maly's Jahresber., **22**; Zaitschek, l. c.; Wróblewsky, "Beiträge zur Kenntnis des Frauenkaseins" (Inaug.-Diss. Bern, 1894), and "Ein neuer eiweis-artiger Bestandteil der Milch," Anzeiger der Akad. d. Wiss. in Krakau, 1898; Kobrak, Pflüger's Arch., **80**.

[2] Pfeiffer, Jahrb. f. Kinderheilkunde, **20**, also Maly's Jahresber., **13**; V. Adriance and J. Adriance, A Clinical Report of the Chemical Examination, etc., Archives of Pediatrics, 1897; Camerer and Söldner, Zeitschr. f. Biologie, **33** and **36**. In regard to the composition of woman's milk, see also Biel, Maly's Jahresber., **4**; Christenn, *ibid.*, **7**; Mendes de Leon, *ibid.*, **12**; Gerber, Bull. Soc. chim., **23**; Tolmatscheff, Hoppe-Seyler's Med.-chem. Untersuch., 272.

From a quantitative standpoint, the most essential differences between woman's and cow's milk are as follows: As compared with the quantity of albumin, the quantity of casein is not only absolutely but also relatively smaller in woman's milk than in cow's milk, while the latter is poorer in milk-sugar. Human milk is richer in lecithin, at least relatively to the amount of protein. BUROW found 0.49–0.58 p. m. lecithin in cow's milk and 0.58 p. m. in woman's milk, which corresponds to 1.40 per cent for the first milk and 3.05 per cent for the second, calculated on the percentage of protein. According to KOCH human milk and cow's milk contain lecithin as well as cephalin. The total quantity of both bodies in human milk was 0.78 p. m. and in cow's milk 0.72–0.86 p. m. The quantity of nucleon is greater in woman's milk. According to WITTMAACK cow's milk contains 0.566 p. m. nucleon, and woman's milk 1.24 p. m., and according to VALENTI the quantity of nucleon in human milk is indeed still higher. SIEGFRIED finds that the nucleon phosphorus amounts to 6.0 per cent of the total phosphorus in cow's milk and 41.5 per cent in woman's milk, and also that in human milk the phosphorus is nearly entirely in organic combination. Because of the large amount of casein (and calcium phosphate) cow's milk is much richer in phosphorus than human milk. The relation P_2O_5:N, according to SCHLOSSMANN,[1] is equal to 1:5.4 in human milk and 1:2.7 in cow's milk. Woman's milk is poorer in mineral bodies, especially lime, and it contains only one-sixth of the quantity of lime as compared with cow's milk. The mineral constituents of human milk are better assimilated by the organism of the nursing child than those of cow's milk. Human milk is claimed to be also poorer in citric acid (SCHEIBE[2]), although this is not an essential difference.

Another difference between woman's milk and other varieties of milk is UMIKOFF's reaction, which seems to depend upon the quantitative composition, especially the relation between the milk-sugar, citric acid, lime, and iron (SIEBER[3]). This reaction consists in treating 5 c.c. of woman's milk with 2.5 c.c. ammonia (10 per cent) and heating to 60° C. for 15–20 minutes, when the mixture becomes violet-red. Cow's milk gives a yellowish-brown color when thus treated.

According to RUBNER woman's milk contains about 3 p. m. soaps, but this could not be substantiated by CAMERER and SÖLDNER. According to them woman's milk contains no soaps, or at least only very small amounts. They also found the quantity of urea nitrogen in woman's milk to be 0.11–0.12 p. m., although SCHÖNDORFF[4] found nearly twice this amount, namely, 0.23 p. m.

In regard to the quantity of *mineral bodies* in woman's milk we have the analyses of several investigators, especially of BUNGE (analyses *A* and *B*) and of SÖLDNER and CAMERER (analysis *C*)[1]. BUNGE analyzed the milk of a woman, fourteen days after delivery, whose diet contained very little common salt for four days previous to the analysis (*A*), and again three days later after a daily addition of 30 grams of NaCl to the food (*B*). The figures are in 1000 parts of the milk:

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[1] Burow, Zeitschr. f. physiol. Chem., **30**; Koch, *ibid.*, **47**; Wittmaack, *ibid.*, **22**; Siegfried, *ibid.*, **22**; Valenti, Biochem. Centralbl., **4**; Schlossmann, Arch. f. Kinderheilkunde, **40**.

[2] Maly's Jahresber., **21**.

[3] Zeitschr. f. physiol. Chem., **30**.

[4] Rubner, Zeitschr. f. Biologie, **36**; Camerer and Söldner, *ibid.*, **39**; Schöndorff, Pflüger's Arch., **81**.

	A.	*B.*	*C.*
K_2O	0.780	0.703	0.884
Na_2O	0.232	0.257	0.357
CaO	0.328	0.343	0.378
MgO	0.064	0.065	0.053
Fe_2O_3	0.004	0.006	0.002
P_2O_5	0.473	0.469	0.310
Cl	0.438	0.445	0.591

The relationship of the two bodies potassium and sodium, to each other may, according to BUNGE, vary considerably (1.3–4.4 equivalents of potash to 1 of soda). By the addition of salt to the food the quantity of sodium and chlorine in the milk increases, while the quantity of potassium decreases. DE LANGE found more Na than K in the milk at the beginning of lactation. JOLLES and FRIEDJUNG found on an average 5.9 milligrams of iron per liter of woman's milk. CAMERER and SÖLDNER[2] find about the same amount, namely, 10–20 milligrams $Fe_2O_3 = 3.5–7$ milligrams iron in 1000 grams human milk.

The gases of woman's milk have been investigated by KÜLZ.[3] He found 1.07–1.44 c.c. of oxygen, 2.35–2.87 c.c. of carbon dioxide, and 3.37–3.81 c.c. of nitrogen in 100 c.c. of milk.

The proper treatment of cow's milk by diluting it with water and by certain additions in order to render it a proper substitute for woman's milk in the nourishment of children cannot be determined before the difference in the protein bodies of these two kinds of milk has been completely studied.

The **colostrum** has a higher specific gravity, 1.040–1.060, a greater quantity of coagulable proteins, and a deeper yellow color than ordinary woman's milk. Even a few days after delivery the color becomes less yellow, the quantity of albumin less, and the number of colostrum-corpuscles diminishes.

We have the older analyses of CLEMM[4] and the recent investigations of PFEIFFER, V. and J. ADRIANCE, CAMERER and SÖLDNER on the changes in the composition of milk after delivery. It follows, as a unanimous result from these investigations, that the quantity of protein, which amounts to more the first two days, sometimes to more than 30 p. m. at first, rather quickly and then more gradually diminishes as long as the lactation continues, so that in the third week it equals about 10–18 p. m. Like the protein substances, the mineral bodies also gradually decrease. The quantity of fat shows no regular or constant variation during lactation, while the lactose, especially according to the observations of V. and J. ADRIANCE (120 analyses), increases rather quickly the first days and then only slowly until the end of lactation. The analyses of PFEIFFER, CAMERER and SÖLDNER also show an increase in the quantity of milk-sugar.

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[1] Bunge, Zeitschr. f. Biologie, 10; Camerer and Söldner, *ibid.*, 39 and 44.

[2] De Lange, Maly's Jahresber., 27; Jolles and Friedjung, Arch. f. exp. Path. u. Pharm., 46; Camerer and Söldner, Zeitschr. f. Biologie, 46.

[3] Zeitschr. f. Biologie, 32.

[4] See Hoppe-Seyler Physiol. Chem., 734.

The two mammary glands of the same woman may yield somewhat different milk, as shown by SOURDAT and later by BRUNNER.[1] Likewise the different portions of milk from the same milking may have varying composition. The first portions are always poorer in fat.

According to L'HÉRITIER and to VERNOIS and BECQUEREL, the milk of blondes contains less casein than that of brunettes, a difference which TOLMATSCHEFF[2] could not substantiate. Women of delicate constitutions yield a milk richer in solids, especially in casein, than women with strong constitutions (V. and B.).

According to VERNOIS and BECQUEREL, the age of the woman has an effect on the composition of the milk, so that we find a greater quantity of proteins and fat in women 15–20 years old and a smaller quantity of sugar. The smallest quantity of proteins and the greatest quantity of sugar are found at 20 or from 25 to 30 years of age. According to VERNOIS and BECQUEREL, the milk with the first-born is richer in water—with a proportionate diminution of casein, sugar, and fat—than after several deliveries.

The influence of menstruation seems to slightly diminish the milk-sugar and to considerably increase the fat and casein (VERNOIS and BECQUEREL).

Witch's milk is the secretion of the mammary glands of new-born children of both sexes immediately after birth. This secretion has from a qualitative standpoint the same constitution as milk, but may show important differences and variations from a quantitative point of view. SCHLOSSBERGER and HAUFF, GUBLER and QUEVENNE, and v. GENSER[3] have made analyses of this milk and give the following results: 10.5–28 p. m. proteins, 8.2–14.6 p. m. fat, and 9–60 p. m. sugar.

As milk is the only form of nourishment during a certain period of the life of man and mammals, it must contain all the nutriment necessary for life. This fact is shown by the milk containing respresentatives of the three chief groups of organic nutritive substances—proteins, carbohydrates, and fat; and all milk seems to contain without doubt also some lecithin and nucleon. The mineral bodies in milk must also occur in proper proportions, and on this point the experiments of BUNGE on dogs are of special interest. He found that the mineral bodies of the milk occur in about the same relative proportion as they do in the body of the sucking animal. Bunge[1] found in 1000 parts of the ash the following results (*A* represents results from the new-born dog, and *B* the milk from the bitch):

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[1] Sourdat, Compt. rend., **71**; Brunner, Pflüger's Arch., **7**.

[2] l'Héritier, cited from Hoppe-Seyler, Physiol. Chem., 738; Vernois and Becquerel, Du lait chez la femme dans l'état de santé, etc. (Paris, 1853); Tolmatscheff, Hoppe-Seyler, Med.-chem. Untersuch., 272.

[3] Schlossberger and Hauff, Annal. d. Chem. u. Pharm., **96**; Gubler and Quevenne, cited from Hoppe-Seyler's Physiol. Chem., 723; v. Genser, *ibid.*

	A.	*B.*
K_2O	114.2	149.8
Na_2O	106.4	88.0
CaO	295.2	272.4
MgO	18.2	15.4
Fe_2O_3	7.2	1.2
P_2O_5	394.2	342.2
Cl	83.5	169.0

Bunge explains the fact that the milk-ash is richer in potash and poorer in soda than the new-born animal by saying that in the growing animal the ash of the muscles rich in potash relatively increases and the cartilage rich in soda relatively decreases. In regard to the amount of iron we find an unexpected condition, the ash of the new-born animal containing six times as much as the milk-ash. This condition Bunge explains by the fact founded on his and Zalesky's experiments, that the quantity of iron in the entire organism is highest at birth. The new-born has therefore its own supply of iron for the growth of its organs even at birth.

The investigations of Hugounenq, de Lange, Camerer and Söldner[2] have shown that in man the conditions are different from those in animals, as the ash of the child has an entirely different composition as compared to the milk. As an example the following analyses are given (of Camerer and Söldner). (*A*, the ash of the sucking infant, and *B*, the ash of the milk.) The results are in 1000 parts of the ash.

	A.	*B.*
K_2O	78	314
Na_2O	91	119
CaO	361	164
MgO	9	26
Fe_2O_3	8	6
P_2O_5	389	135
Cl	77	200

We cannot therefore state as a definite fact that the composition of the ash of the sucking young and the ash of the corresponding milk coincide. Bunge[3] nevertheless claims that the composition of the ash of the sucking young of various mammals is nearly the same, but that the ash of the milk differs from the ash of the young in so far as the slower the young grows the richer it is in alkali chlorides and relatively poorer in phosphates and lime-salts. The constituents of the ash have two functions to perform, namely, the building up of the tissues and secondly the preparation of the excreta, especially the urine. The faster the young grows the more is the first in evidence, while the slower it develops, the second is prominent.

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[1] Zeitschr. f. physiol. Chem., **13**.

[2] Hugounenq, Compt. rend., **128**; de Lange, Zeitschr. f. Biologie, **40**; Camerer and Söldner, *ibid.*, **39**, **40**, and **44**.

[3] Bunge, "Die zunehmende Unfähigkeit der Frauen ihre Kinder zu stillen," München, 1900, cited by Camerer, Zeitschr. f. Biologie, **40**.

The quantity of mineral bodies in the milk, and especially the amount of lime and phosphoric acid, as shown by Bunge and Pröscher and Pagès, stands in close relationship to the rapidity of growth, because the amount of these mineral constituents in the milk is greater in animals which grow and develop quickly than in those which grow only slowly. A similar relationship exists also, as shown by the researches of Pröscher, and especially of Abderhalden,[1] between the quantity of protein in the milk and the rapidity of development of the sucking young. The amount of protein is greater in the milk the quicker the animal develops.

The *influence of the food* on the composition of the milk is of interest from many points of view and has been the subject of many investigations. From these we learn that in human beings as well as in animals an insufficient diet decreases the quantity of milk and the quantity of solids, while abundant food increases both. From the observations of Decaisne[2] on nursing women during the siege of Paris in 1871, the amount of casein, fat, sugar, and salts, but especially the fat, was found to decrease with insufficient food, while the quantity of lactalbumin was found to be somewhat increased. Food rich in proteins increases the quantity of milk, and also the solids contained, especially the fat, according to most statements. The quantity of sugar in woman's milk is found by certain investigators to be increased after food rich in proteins, while others claim it is diminished. A diet rich in fat may, as the researches of Soxhlet and many others[3] have shown, cause a marked increase in the fat of the milk when the fat partaken is in a readily digestible and assimilable form. The presence of large quantities of carbohydrates in the food seems to cause no constant, direct action on the quantity of the milk constituents.[4] In carnivora, as shown by Ssubotin,[5] the secretion of milk-sugar proceeds uninterruptedly on a diet consisting exclusively of lean meat. Watery food gives a milk containing an excess of water and having little value. In the milk from cows which were fed on distillers' grain COMMAILLE [1] found 906.5 p. m. water, 26.4 p. m. casein, 4.3 p. m. albumin, 18.2 p. m. fat, and 33.8 p. m. sugar. Such milk has sometimes a peculiar sharp after-taste, although not always.[2]

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[1] Pröscher, Zeitschr. f. physiol. Chem., **24**; Abderhalden, *ibid.*, **27**; Pagès, Arch. de Physiol. (5), **7**.

[2] Cited from Hoppe-Seyler, l. c., 739.

[3] See Maly's Jahresber., **26**. See also Basch, Ergebnisse der Physiologie, **2**, Abt. 1.

[4] In regard to the literature on the action of various foods on woman's milk, see Zalesky, "Ueber die Einwirkung der Nahrung auf die Zusammensetzung und Nahrhaftigkeit der Frauenmilch," Berlin. klin. Wochenschr., 1888, which also contains the literature on the importance of diet on the composition of other kinds of milk. In regard to the extensive literature on the influence of various foods on the milk production of animals, see König, Chem. d. menschl. Nahrungs und Genussmittel, 3. Aufl., **1**, 298. See also Maly's Jahresber., **29**, **30**, **31**, and Morgen, Beger and Fingerling, Landw. Versuchsst., **61**.

[5] Centralbl. f. d. med. Wissensch., 1866, 337.

Chemistry of Milk-secretion. That the constituents which occur actually dissolved in milk pass into the secretion not alone by filtration or diffusion, but more likely are secreted by a specific secretory activity of the glandular elements, is shown by the fact that milk-sugar, which is not found in the blood, is to all appearances formed in the glands themselves. A further proof lies in the fact that the lactalbumin is not identical with seralbumin; and lastly, as BUNGE [3] has shown, the mineral bodies secreted by the milk are in quite different proportions from those in the blood-serum.

Little is known in regard to the formation and secretion of the specific constituents of milk. The older theory, that the casein was produced from the lactalbumin by the action of an enzyme, is incorrect and originated probably from mistaking an alkali albuminate for casein. Better founded is the statement that the casein originates from the protoplasm of the gland-cells. There does not seem to be any doubt that the protoplasm of the cells takes part in the secretion in such a manner that it becomes itself a constituent of the secretion, and this also agrees with HEIDENHAIN'S [4] views. According to BASCH'S researches the casein is formed in the mammary gland by the nucleic acid of the nucleus being set free and uniting intra-alveolar with the transudated serum, thus forming a nucleoalbumin, the casein. The untenableness of this view has been shown by LÖBISCH, and the investigations of HILDEBRANDT [5] upon the proteolytic enzyme of the mammary gland and the autolysis of the gland have not given any clue as to the mode of formation of casein.

That the milk-fat is produced by a formation of fat in the protoplasm, and that the fat-globules are set free by their destruction, is a generally admitted opinion, which, however, does not exclude the possibility that the fat is in part taken up by the glands from the blood and eliminated with its secretion. That the fats of the food can pass into the milk follows from the investigations of WINTERNITZ, as he has been able to detect the passage of iodized fats in the milk. JANTZEN has shown that after feeding iodized casein, the milk-fat of goats contained a little iodine, which indicates that the iodized milk-fat could also have a different origin. As a contamination of the casein fed with iodized fat was not excluded in these experiments, they do not seem to modify the proof of the investigations of WINTERNITZ and others (CASPARI, PARASCHTSHUK[1]). The abundant quantities of iodized fat which were eliminated with the milk in these cases without doubt depend, at least in great part, upon the iodized fat of the food, hence it cannot be said that all of the milk-fat containing iodine was unchanged iodized fat of the food. The investigations of SPAMPANI and DADDI, PARASCHTSCHUK, GOGITIDSE and others on the passage of foreign fats into the milk also indicate the passage of the fat of the food into the milk, although we are still uncertain on this point. According to SOXHLET the fat of the food does not pass into the milk directly, but is destroyed in place of the body-fat, which then becomes available and is, as it were, pushed into the milk. HENRIQUES and HANSEN could not detect any mentionable quantity of linseed-oil in the milk after feeding with this oil; the milk-fat was not normal, but had a higher iodine equivalent and a higher melting-point, from which they also concluded that a transformation of the food-fat in the glandular cells is possible. The experiments of GOGITIDSE[2] with soaps also speak for the fact that the mammary glands have the property of forming fats by synthesis from their components. As a formation of fat from carbohydrates in the animal organism is at the present day considered as positively proved, it is likewise possible that the milk-glands also produce fats from the carbohydrates brought to them by the blood. It is a well-known fact that an animal gives off for a long time, daily, considerably more fat in the milk than it receives as food, and this proves that at least a part of the fat secreted by the milk is produced from proteins or carbohydrates, or perhaps from both. The question as to how far this fat is produced directly in the milk-glands, or from other organs and tissues, and brought to the gland by means of the blood, cannot be decided.

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[1] Cited from König, 2, 235.

[2] See Beck, Maly's Jahresber., 25.

[3] Lehrbuch d. physiol. und pathol. Chem., 3. Aufl., 93.

[4] Hermann's Handbuch, 5, Teil 1, 380.

[5] Basch, Jahrb. f. Kinderheilkunde, 1898; Hildebrandt, Hofmeister's Beiträge, 5; Löbisch, *ibid.*, 8.

The origin of milk-sugar is not known. MÜNTZ calls attention to the fact that a number of very widely diffused bodies in the vegetable kingdom—vegetable mucilage, gums, pectin bodies—yield galactose as a product of decomposition, and he believes, therefore, that milk-sugar may be formed in herbivora by a synthesis from dextrose and galactose. This origin of milk-sugar does not apply to carnivora, as they produce milk-sugar when fed on food consisting entirely of lean meat. The observations of BERT and THIERFELDER[1] that a mother-substance of the milk-sugar, a saccharogen, occurs in the glands cannot give further explanation as to the formation of milk-sugar, as the nature of this mother-substance is still unknown. As the animal body has undoubtedly the power of converting one variety of sugar into another, the origin of the milk-sugar can be sought simply in the dextrose introduced as food or formed in the body. Certain observations indicate such an origin, among others those of PORCHER,[2] who found that dextrose appeared in the urine after delivery when the mammary glands of the goat had previously been extirpated. This glycosuria is explained simply by the fact that the lactose-forming action of the gland was removed at the time of delivery, when large amounts of dextrose were produced.

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[1] Winternitz, Zeitschr. f. physiol. Chem., **24**; Jantzen, Centralbl. f. Physiol., **15**; Caspari, Arch. f. (Anat. u.) Physiol., 1899, Supplbd. and Zeitschr. f. Biologie, **46**; Paraschtschuk, Chem. Centralbl., 1903, **1**.

[2] Spampani and Daddi, Maly's Jahresber., **26**; Henriques and Hansen, *ibid.*, **29**; Gogitidse, Zeitschr. f. Biologie, **45** and **46**. See also Basch, Ergebnisse d. Physiol., **2**, Abt. 1.

The passage of foreign substances into the milk stands in close connection with the chemical processes of milk secretion.

It is a well-known fact that milk acquires a foreign taste from the food of the animal, which is in itself a proof that foreign bodies pass into the milk. This fact becomes of special importance in reference to such injurious substances as may be introduced into the organism of the nursing child by means of the milk.

Among these substances may be mentioned opium and morphine, which after large doses pass into the milk and act on the child. Alcohol may also pass into the milk, but probably not in such quantities as to have any direct action on the nursing child.[3] Alcohol is claimed to have been detected in the milk after feeding cows with brewer's grains.

Among inorganic bodies, iodine, arsenic, bismuth, antimony, zinc, lead, mercury, and iron have been found in milk. In icterus neither bile-acids nor bile-pigments pass into the milk.

Under diseased conditions no constant change has been found in woman's milk. In isolated cases SCHLOSSBERGER, JOLY and FILHOL[4] have observed indeed a markedly abnormal composition, but no positive conclusion can be derived therefrom.

The changes in cow's milk in disease have been little studied. In tuberculosis of the udder STORCH[5] found tubercle bacilli in the milk, and he also noted that the milk became more and more diluted, during the disease, with a serous liquid similar to blood-serum, so that the glands finally, instead of yielding milk, gave only blood-serum or a serous fluid. HUSSON[6] found that milk from murrain cows contained more proteins but considerably less fat and (in severe cases) less sugar than normal milk.

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[1] Müntz, Compt. rend., **102**; Bert and Thierfelder, foot-note 3, p. 514.

[2] Compt. rend., **138** and **141**.

[3] See Klingemann, Virchow's Arch., **126**, and Rosemann, Pflüger's Arch., **78**.

[4] Schlossberger, Annal. d. Chem. u. Pharm., **96**; Joly and Filhol, cited from v. Gorup-Besanez, Lehrb., 4. Aufl., 438.

[5] See Bang, Om Tuberkulose i Koens Yver og om tuberkulös Mälk, Nord. med. Arkiv, **16**, and also Maly's Jahresber., **14**, 170; Storch Maly's Jahresber., **14**.

[6] Compt. rend., **73**.

The milk may be blue or red in color, due to the development of microorganisms.

The formation of concrements in the exit-passages of the cow's udder is often observed. These consist chiefly of calcium carbonate, or of carbonate and phosphate with only a small amount of organic substances.

CHAPTER XV.

URINE.

URINE is the most important excretion of the animal organism; it is the means of eliminating the nitrogenous metabolic products, also the water and the soluble mineral substances; and in many cases it furnishes important data relative to the metabolism, quantitatively by its variation, and qualitatively by the appearance of foreign bodies in the excretion. Moreover in many cases we are able from the chemical or morphological constituents which the urine abstracts from the kidneys, ureter, bladder, and urethra to judge of the condition of these organs; and lastly, urinary analysis affords an excellent means of deciding the question as to how certain medicinal agents or other foreign substances introduced into the organism are absorbed and chemically changed. In this respect especially urinary analysis has furnished very important particulars in regard to the nature of the chemical processes taking place within the organism, and it is therefore not only an important aid in diagnosis to the physician, but it is also of the greatest importance to the toxicologist and the physiological chemist.

In studying the secretions and excretions the relationship must be sought between the chemical structure of the secreting organ and the chemical composition of its secreted products. Investigations with respect to the kidneys and the urine have led to very few results from this standpoint. Although the anatomical relation of the kidneys has been carefully studied, their chemical composition has not been the subject of thorough analytical research. In cases in which a chemical investigation of the kidneys has been undertaken, it has been in general only of the organ as such, and not of the different anatomical parts. An enumeration of the chemical constituents of the kidneys known at the present time can, therefore, have only a secondary value.

In the kidneys we find proteins of different kinds. According to HALLIBURTON the kidneys do not contain any albumin, but only a *globulin* and a *nucleoproteid*. The globulin coagulates at about 52° C., and the nucleoproteid contains 0.37 per cent phosphorus. According to L. LEIBERMANN the kidneys contain a *lecithalbumin*, and he ascribes to this body a special importance in the secretion of acid urines. The kidneys also contain, according to LÖNNBERG, a *mucin-like substance.* This substance yields no reducing body on boiling with acids and belongs chiefly to the papillæ, and is, according to LÖNNBERG, a nucleoalbumin (nucleoproteid?). The cortical substance is richer in another nucleoalbumin (nucleoproteid) unlike mucin. It has not been decided what relationship this last substance bears to HALLIBURTON'S nucleoproteid. The nucleic acid obtained by MANDEL and LEVENE from beef kidneys yielded guanine, adenine, thymine, and cytosine on cleavage. According to MÖRNER[1] *chondroitin-sulphuric acid* occurs as traces. MANDEL and LEVENE[2] have also obtained *glucothionic acid* from the kidneys. *Fat* occurs only in very small amounts in the cells of the tortuous urinary passages. Among the extractive bodies of the kidneys one finds *purine bases*, also *urea*, *uric acid* (traces), *glycogen*, *leucine*, *inosite*, *taurine*, and *cystine* (in ox-kidneys). The quantitative analyses of the kidneys thus far made possess little interest. OIDTMANN[3] found 810.94 p. m. water, 179.16 p. m. organic and 0.99 p. m. inorganic substance in the kidney of an old woman.

The fluid collected under pathological conditions, as in hydronephrosis, is thin with a variable but generally low specific gravity. Usually it is straw-yellow or paler in color, and sometimes colorless. Most frequently it is clear, or only faintly cloudy from white blood-corpuscles and epithelium-cells; in a few cases it is so rich in form-elements that it appears like pus. Protein occurs generally in small amounts; occasionally it is entirely absent, but in a few rare cases the amount is nearly as large as in the blood-serum. Urea occurs sometimes in considerable amounts when the parenchyma of the kidneys is only in part atrophied; in complete atrophy the urea may be entirely absent.

I. Physical Properties of Urine.

Consistency, Transparency, Odor, and Taste of Urine. Under physiological conditions urine is a thin liquid and gives, when shaken with air, a froth which quickly subsides. Human urine, or urine from carnivora, which is habitually acid, appears clear and transparent, often faintly fluorescent, immediately after voiding. When allowed to stand for a little while human urine shows a light cloud (*nubecula*), which consists of the so-called "mucus," and generally also contains a few epithelium cells, mucus-corpuscles, and urate-granules. The presence of a larger quantity of urates renders the urine cloudy, and a clay-yellow, yellowish-brown, rose-colored, or often brick-red precipitate (*sedimentum lateritium*) settles on cooling, because of the greater insolubility of the urates at the ordinary temperature than at the temperature of the body. This cloudiness disappears on gently warming. In new-born infants the cloudiness of the urine during the first 4–5 days is due to epithelium, mucus-corpuscles, uric acid, and urates. The urine of herbivora, which is habitually neutral or alkaline in reaction, is very cloudy on account of the carbonates of the alkaline earths present. Human urine may sometimes be alkaline under physiological conditions. In this case it is cloudy, due to the earthy phosphates, and this cloudiness does not disappear on warming, differing in this respect from the *sedimentum lateritium.* Urine has a salty and faintly bitter taste produced by sodium chloride and urea. The odor of urine is peculiarly aromatic; the bodies which produce this odor are unknown.

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[1] Halliburton, Journ. of Physiol., **13**, Suppl., and **18**; Liebermann, Pflüger's Arch., **50** and **54**; Lönnberg, see Maly's Jahresber., **20**; Mandel and Levene, Zeitschr. f. physiol. Chem., **47**; Mörner, Skand. Arch. f. Physiol., **6**.

[2] Zeitschr. f. physiol. Chem., **45**.

[3] Cited from v. Gorup-Besanez, Lehrbuch, 4. Aufl., 732.

The **color** of urine is normally pale yellow when the specific gravity is 1.020. The color otherwise depends on the concentration of the urine and varies from pale straw-yellow, when the urine contains small amounts of solids, to a dark reddish yellow or reddish brown in stronger concentration. As a rule the intensity of the color corresponds to the concentration, but under pathological conditions exceptions occur such as is found in diabetic urine, which contains a large amount of solids and has a high specific gravity and a pale-yellow color.

The **reaction** of urine depends essentially upon the composition of the food. The carnivora, as a rule, void an acid, the herbivora, a neutral or alkaline urine. If a carnivore is put upon a vegetable diet, its urine may become less acid or neutral, while the reverse occurs when an herbivore is starved, that is, when it lives upon its own flesh, as then the urine voided is acid.

The urine of a healthy man on a mixed diet has an *acid reaction*, and the sum of the acid equivalents is greater than the sum of the basic equivalents. This depends upon the fact that in the physiological combustion of neutral substances (proteins and others) within the organism, acids are produced, chiefly sulphuric acid, but also phosphoric and organic acids, such as hippuric, uric, and oxalic acids, aromatic oxyacids, and others. From this it follows that the acid reaction is not due to one acid alone. The ordinary view that the acid reaction is due chiefly to dihydrogen phosphates is therefore not true. The various acids take part in the acid reaction in proportion to their dissociation, since, according to the ion theory, the acid reaction of a mixture is dependent upon the number of hydrogen ions present.

The composition of the food is not the only influence which affects the degree of acidity of human urine. For example, after taking food, at the beginning of digestion, when a larger amount of gastric juice containing hydrochloric acid is secreted, the urine may be neutral or even alkaline.[1] The statements of various investigators are rather contradictory in regard to the time of the appearance of the maximum and minimum of the acidity, which may in part be explained by the varying individuality and conditions of life of the persons investigated. It has not infrequently been observed that perfectly healthy persons in the morning void a neutral or alkaline urine which is cloudy from earthy phosphates. The effect of muscular activity on the acidity of urine has not been positively determined. According to HOFFMANN, RINGSTEDT, ODDI and TARULLI, and VOZÁRIK muscular work raises the degree of acidity, but ADUCCO[1] claims that it decreases it. Abundant perspiration reduces the acidity (HOFFMANN).

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[1] Contradictory statements are found in Linossier, Maly's Jahresber., 27.

In man and especially in carnivora it seems that the degree of acidity of the urine cannot be increased above a certain point, even though mineral acids or organic acids which are burnt up with difficulty are ingested in large quantities. When the supply of carbonates of the fixed alkalies stored up in the organism for this purpose is not sufficient to combine with the excess of acid, then ammonia is split off from the proteins or their decomposition products, and this excess of acid combines therewith, forming ammonium salts, which pass into the urine. In herbivora such a combination of the excess of acid with ammonia does not seem to take place, or not to the same extent, and therefore herbivora soon die when acids are given. This is true at least for rabbits, while according to BAER[2] this power of increasing the elimination of ammonia exists also in the goat, monkey, and pig, hence no definite difference in this regard exists between herbivora and carnivora. Nevertheless the degree of acidity of human urine may be easily diminished so that the reaction becomes neutral or alkaline. This occurs after the taking of carbonates of the fixed alkalies or of such alkali salts of vegetable acids—tartaric acid, citric acid, and malic acid—as are easily burnt into carbonates in the organism. Under pathological conditions, as in the absorption of alkaline transudates, or the alkaline fermentation within the bladder, the urine may become alkaline.

A urine with an alkaline reaction caused by fixed alkalies has a very different diagnostic value from one whose alkaline reaction is caused by the presence of ammonium carbonate. In the latter case we have to deal with a decomposition of the urea of the urine by the action of micro-organisms.

If one wishes to determine whether the alkaline reaction of the urine is due to ammonia or to fixed alkalies, a piece of red litmus paper is dipped into the urine and allowed to dry exposed to the air or to a gentle heat. If the alkaline reaction is due to ammonia, the paper becomes red again; but if it is caused by fixed alkalies, it remains blue.

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[1] Hoffmann, see Maly's Jahresber., **14**; Ringstedt, *ibid.*, **20**; Oddi and Tarulli, *ibid.*, **24**; Aducco, *ibid.*, **17**; Vozárik, Pflüger's Arch., **111**.

[2] See Winterberg, Zeitschr. f. physiol. Chem., **25**, and T. Baer, Arch. f. exp. Path. u. Pharm., **54**.

Determination of the Acidity. As the quantity of phosphoric acid present as dihydrogen salt, as above stated, cannot be used as a measure of the acidity, all the older methods suggested for the estimation of this portion of the phosphoric acid are not suited for acidity determinations. We now determine the acidity simply by acidimetric methods, titrating with N/10 caustic alkali, using phenolphthalein as an indicator (Naegeli, Höber, Folin). On account of the color of the urine and the presence of ammonium salts and alkaline earths, this method cannot yield entirely exact results. The greatest error depends upon the alkaline earths, which, on titration with caustic alkali, precipitate as earthy phosphates in variable amounts and of variable composition. This error can be prevented, according to Folin, by the addition of neutral potassium oxalate, which precipitates the lime, and in this way the disturbing action of the ammonium salts is also inhibited. Perfectly acccurate results are not obtained by this method, but it is the best of those which have been suggested.

It is performed as follows: 25 c.c. of urine are placed in an Erlenmeyer flask (about 200 c.c. capacity), treated with 1–2 drops of ½ per cent phenolphthalein solution, and shaken with 15–20 grams of powdered potassium oxalate and immediately titrated with N/10 caustic soda with constant shaking until a pronounced pale-rose color appears. Vozárik[1] titrates the diluted urine without the addition of oxalate and uses phenolphthalein as indicator.

The acidity, as determined by titration, varies considerably under physiological conditions, but calculated as hydrochloric acid it amounts to about 1.5–2.3 grams in man in the 24 hours.

By titration we learn the amount of hydrogen present which can be substituted by a metal, i.e., the acidity in the ordinary older sense, but not the true acidity, the ion acidity, which is given by the concentration of the hydrogen ions of the urine. For similar reasons, as indicated previously in treating of the alkalinity of the blood-serum (page 191), the ion acidity cannot be determined by titration, while it can be determined according to the principle of the electrometric gas-chain method as there given. Such estimations have been made by v. Rhorer and by Höber.[2] For normal urine v. Rhörer found as a minimum 4×10^{-7}, as a maximum 76×10^{-7}, and as an average 30×10^{-7}. Höber found 4.7×10^{-7}, $100\times^{-7}$, and 49×10^{-7}, respectively. On an average the urine contains therefore 30–50 grams of hydrogen ions in 10 million liters, and as in the same quantity of purest water there is contained in round numbers 1 gram of hydrogen ions, the urine contains, therefore, 30–50 times as many hydrogen ions as the water. From HÖBER's investigations it also follows that no direct relationship exists between the titration acidity and the ion acidity, and that the extent of these two acidities may be independent of each other.

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[1] Naegeli, Zeitschr. f. physiol. Chem., **30**; Höber, Hofmeister's Beiträge, **3**; Folin, Amer. Journ. of Physiol., **9**; Vozárik, l. c.

[2] v. Rhörer, Pflüger's Arch., **86**; Höber, l. c.

The **osmotic pressure** of the urine varies considerably even under physiological conditions. The limits for the freezing-point depression has been found by a number of investigators to be $\Delta = 0.87° - 2.71°$ C.[1] After partaking of considerable water it may be markedly lower, and on diminished supply of water it may be considerably higher.

According to BUGARSKY a certain relationship exists between the freezing-point depression and the specific gravity, namely, $\frac{\Delta}{s-1} = \text{constant} = 75$. This equation, where s represents the specific gravity, has no general application, and according to STEYRER [2] is only approximate for normal urines. The validity of the relationship found by BUGARSKY between the electrical conductivity and the ash content of the urine, seems also to require further proof.

The **specific gravity** of urine, which is dependent upon the relationship existing between the quantity of water secreted and the solid urinary constituents, especially the urea and sodium chloride, may vary considerably, but is generally 1.017–1 020. After drinking large quantities of water it may fall to 1.002, while after profuse perspiration or after drinking very little water it may rise to 1.035–1.040. In new-born infants the specific gravity is low, 1.007–1.005. The determination of the specific gravity is an important means of learning the average amount of solids eliminated from the organism in the urine, and on this account the determination becomes of true value only when at the same time the quantity of urine voided in a given time is determined. The different portions of urine voided in the course of the twenty-four hours are collected, mixed together, the total quantity measured, and then the specific gravity taken.

The *determination of the specific gravity* is most accurately obtained with the pycnometer. For ordinary cases the specific gravity may be determined with sufficient accuracy by means of areometers. The areometers found in the trade, or *urinometers*, are graduated from 1.000 to 1.040; for exact observations it is better to use two urinometers, one graduated from 1.000 to 1.020, and the other from 1.020 to 1.040.

To determine the specific gravity of urine, if necessary filter the urine, or if it contains a urate sediment, first dissolve it by gentle heat, then pour the clear urine into a dry cylinder, avoiding the formation of froth. Air-bubbles or froth, when present, must be removed with a glass rod or filter-paper. The cylinder, which should be about four-fifths full, must be wide enough to allow the urinometer to swim freely in the liquid without touching the sides. The cylinder and urinometer should both be dry or previously washed with the urine. On reading, the eye is brought on a level with the lower meniscus—which occurs when the surface of the liquid and the lower limb of the meniscus coincide; the reading is then made from the point where this curved line coincides with the scale of the urinometer. If the eye is not in the same horizontal plane with the convex line of the meniscus, but is too high or too low, the surface of the liquid assumes the shape of an ellipse, and the reading in this position is incorrect. Before reading, press the urinometer gently down into the liquid and then allow it to rise, and wait until it is at rest.

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[1] See Strauss, Zeitschr. f. klin. Med., **47**.

[2] Bugarsky, Pflüger's Arch., **68**; Steyrer, Hofmeister's Beiträge, **2**.

Each urinometer is graduated for a certain temperature, which, at least in the case of the better ones, is marked on the instrument. If the urine is not at the proper temperature, the following corrections must be made: For every three degrees above the normal temperature one unit of the last order is added to the reading, and for every three degrees below the normal temperature one unit (as above) is subtracted from the specific gravity observed. For example, when a urinometer graduated for 15° C. shows a specific gravity of 1.017 at 24° C., then the specific gravity at 15° C. = 1.017 + 0.003 = 1.020.

When great exactitude is required, as, for instance, a determination to the fourth decimal point, we make use of a urinometer constructed by LOHNSTEIN.[1] JOLLES[2] has also devised a small urinometer for the determination of the specific gravity of small amounts of urine, 20–25 c.c. The specific gravity may also be determined by the WESTPHAL hydrostatic balance.

II. Organic Physiological Constituents of Urine.

Urea, $\overset{+}{\mathrm{U}}\mathrm{r}$, $\mathrm{CON_2H_4} = \mathrm{CO} < \begin{matrix}\mathrm{NH_2}\\ \mathrm{NH_2}\end{matrix}$, has been synthetically prepared in several ways, especially, as WÖHLER showed in 1828, by the metameric transformation of ammonium isocyanate: $\mathrm{CO.N.NH_4} \rightarrow \mathrm{CO(NH_2)_2}$. It is also produced by the decomposition or oxidation of certain bodies found in the animal organism, such as purine bodies, creatine, agrinine, other amino-acids, and polypeptides.

Urea is found most abundantly in the urine of carnivora and man, but in smaller quantities in that of herbivora. The quantity in human urine is ordinarily 20–30 p. m. It has also been found in small quantities in the urine of amphibians, fishes, and certain birds. Urea occurs in the perspiration in small quantities, and as traces in the blood and in most of the animal fluids. It also occurs in rather large quantities in the blood, liver, muscle,[3] and bile[4] of sharks. Urea is also found in certain tissues and organs of mammals, especially in the liver and spleen, although only in small amounts. Under pathological conditions, as in obstructed excretion, urea may appear to a considerable extent in the animal fluids and tissues.

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[1] Pflüger's Arch., 59; Chem. Centralbl., 1895, 1, and 1896, 2.

[2] Wien. med. Presse, 1897, No. 8.

[3] v. Schroeder, Zeitschr. f. physiol. Chem., 14.

[4] Hammarsten, *ibid.*, 24.

The quantity of urea which is voided in twenty-four hours on a mixed diet is in a grown man about 30 grams, in women somewhat less. While children void less, the excretion relative to their body weight is greater than in grown persons. The physiological significance of urea lies in the fact that this body forms in man and carnivora, from a quantitative standpoint, the most important nitrogenous end-product of the metabolism of protein bodies. On this account the elimination of urea varies to a great extent with the catabolism of the protein, and above all with the quantity of absorbable proteins in the food ingested. The elimination of urea is greatest after an exclusive meat diet, and lowest, indeed less than during starvation, after the consumption of non-nitrogenous substances, since these diminish the metabolism of the proteins of the body.

If the consumption of the proteins of the body is increased, then the elimination of nitrogen is correspondingly increased. This is found to be the case in fevers, after poisoning with arsenic, antimony, phosphorus, and other protoplasmic poisons, and when there is a diminished supply of oxygen—as in severe and continuous dyspnœa, poisoning with carbon monoxide, hemorrhage, etc. In these cases it used to be considered that the rise in the excretion of nitrogen was due to an increased elimination of urea, because no exact difference was made between the quantity of urea and of total nitrogen in the urine. Recent researches have conclusively demonstrated the untrustworthiness of these observations. Since Pflüger and Bohland have shown that 16 per cent of the total nitrogen of the urine exists under physiological conditions in other compounds, not urea, attention has been called to the relationship of the different nitrogenous constituents of the urine to each other, and it has been found, under pathological conditions, that this relationship may vary considerably, especially in regard to the urea. We have numerous determinations by different investigators, such as Bohland, E. Schultze, Camerer, Voges, Mörner and Sjöqvist, Gumlich, Bödtker,[1] and others, on the relationship of the different nitrogenous constituents to each other in the normal urine of adults. Sjöqvist has made similar determinations on new-born babes from 1 to 7 days old. From all these analyses we obtain the following figures (A for adults and B for new-born babes). Of the total nitrogen there exists:

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[1] Pflüger and Bohland, Pflüger's Arch., **38** and **43**; Bohland, *ibid.*, **43**; Schultze, *ibid.*, **45**; Camerer, Zeitschr. f. Biologie, **24**, **27**, and **28**; Voges, Ueber die Mischung der stickstoffhaltigen Bestandtheile im Harn, etc. (Inaug.-Diss. Berlin, 1892), cited from Maly's Jahresber., **22**; K. Mörner and Sjöqvist, Skand. Arch. f. Physiol., **2**. See also Sjöqvist, Nord. med. Arkiv. 1892, No. 36, and 1894, No. 10; Gumlich, Zeitschr. f. physiol. Chem., **17**; Bödtker, see Maly's Jahresber., **26**.

	A. Per Cent.	B. Per Cent.
Urea	84–91	73–76
Ammonia	2–5	7.8–9.6
Uric acid	1–3	3.0–8.5
Remaining nitrogenous substances (extractives)	7–12	7.3–14.7

The variable relationship between uric acid, ammonia, and urea nitrogen in children and adults is remarkable, since the urine of children is considerably richer in uric acid and ammonia, and considerably poorer in urea, than the urine of adults. The absolute quantity of urea nitrogen in adults amounts to about 10–16 grams per day. In disease the proportion of the nitrogenous substances may be markedly changed, and a decrease in the quantity of urea and an increase in the quantity of ammonia have been observed in certain diseases of the liver. This will be considered in detail in connection with the formation of urea in the liver. It is natural that there should be a diminished formation of urea after a decrease in the ingestion of proteins or in a lowered catabolism. In diseases of the kidneys which disturb or destroy the integrity of the epithelium of the convoluted urinary passages, the elimination of urea is considerably diminished.

Recently by means of PFAUNDLER'S[1] method, by precipitating the urine with phosphotungstic acid and closely studying the precipitate as well as the filtrate, it has been possible to learn further about the division of the nitrogen of the urine. We determine *a*, the total nitrogen; *b*, the nitrogen of the phosphotungstate precipitate; and *c*, the nitrogen in the filtrate from the phosphotungstate precipitate. This last contains the urea, hippuric acid, and other bodies whose nitrogen is ordinarily designated as monamino-acid nitrogen. The urea nitrogen is especially determined. The bodies precipitated by phosphotungstic acid are not all known; but uric acid and purine bases, ammonia, creatinine, pigments, diamino-acids, diamines and ptomaines (if they occur), sulphocyanides, carbamic acid, urine mucoid, and proteid belong to this group. Of these bodies, ammonia, uric acid, creatinine and purine bases are specially determined.

The urea nitrogen is always the greatest part of the total nitrogen, but otherwise the division of the nitrogen undergoes considerable variation. According to v. JACKSCH[2] normal human urine contains from 1.5 to 3 per cent of the total nitrogen as amino-acid nitrogen and 5.16 to 8.5 per cent as ammonia and purine bodies. Other experimenters have obtained different results, and our knowledge on this subject is not sufficient. Very great variations seem to occur not only in the healthy individual, but also and to a greater degree in diseased conditions.[3]

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[1] Pfaundler, Zeitschr. f. physiol. Chem., **30**.

[2] Zeitschr. f. klin. Med., **50**.

[3] See Satta, Hofmeister's Beiträge, **6**, which also gives the literature, and Erben, Zeitschr. f. Heilkunde, **25**.

Formation of Urea in the Organism. The experiments to produce urea directly from proteins by oxidation have led to the formation of some guanidine, but urea has not been obtained positively. On the hydrolysis of proteins arginine has been found among other products, and as it is also produced in tryptic digestion, it is possible that a small portion of the urea is produced in this manner, varying according to the kind of protein (DRECHSEL, KOSSEL, see Chapter II). DRECHSEL claims that about 10 per cent of the urea can be accounted for in this way.

rea from
ginine

The possibility of a formation of urea from arginine has gained in interest since KOSSEL and DAKIN have discovered the presence of an enzyme, *arginase*, in the liver and other organs, which has the power of splitting arginine with the formation of urea. THOMPSON[1] has recently given a direct proof for the formation of urea from arginine. The introduction of arginine into the body of a dog either per os or subcutaneously has in his experiments led to an elimination of urea. While outside of the body only one-half of the nitrogen of arginine is split off as urea and the other half as ornithine, in the above experiments the increase in urea in several instances corresponded to the greater part if not the whole of the nitrogen of the arginine introduced. In these cases, without mentioning that the arginine seemed to raise the nitrogen catabolism, probably also urea was formed from the ornithine. This can be explained by a deamidation of the ornithine and formation of urea from the ammonia and carbon dioxide split off.

ea from
nithine

By the action of alkalies, as above mentioned (Chapter XI), urea may be formed from creatine; still such an origin of urea in the animal body has not thus far been proved.

The amino-acids are considered as special mother-substances of urea. By the researches of SCHULTZEN and NENCKI and SALKOWSKI with leucine and glycocoll, those of STOLTE with several amino-acids, and those of v. KNIERIEM with asparagine, it has been shown that the amino-acids are in part converted into urea in the animal organism. The investigations by SALASKIN with the three amino-acids, glycocoll, leucine, and aspartic acid, have unmistakably shown that the surviving dog-liver, supplied with arterial blood, has the property of transforming the above amino-acids into urea or a closely allied substance. The researches of LOEWI with the "urea-forming" enzyme of the liver, discovered by RICHET, and glycocoll or leucine, as also the researches of ASCOLI,[2] have led to similar results, but it must be remarked that we have no proof as to the identity of the newly formed substance with urea. Nothing can be stated in regard to the extent of formation of amino-acids in the physiological destruction of proteins in the animal body, with the exception of those formed in the intestinal digestion. The possibility of such a formation of urea is beyond dispute. As shown by Abderhalden[1] with Teruuchi and Babkin, the polypeptides, like the amino-acids, can also be converted into urea in the animal body.

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[1] Kossel and Dakin, Zeitschr. f. physiol. Chem., **41**; Thompson, Journ. of Physiol., **32** and **33**.

[2] Schultzen and Nencki, Zeitschr. f. Biologie, 8; v. Knieriem, *ibid.*, **10**; Salkowski, Zeitschr. f. physiol. Chem., 4; Salaskin, *ibid.*, **25**; Loewi, *ibid.*, **25**; Stolte, Hofmeister's Beiträge, 5; Richet, Compt. rend., **118**, and Compt. rend. Soc. biol., **49**; Ascoli, Pflüger's Arch., **72**.

Nothing positive can be said in regard to the manner in which this formation of urea occurs; but it is admitted that it is partly a formation from ammonia and partly from carbamic acid.

The possibility of a formation of urea from ammonia has been positively shown. Thus the researches of v. Knieriem, Salkowski, Feder, I. Munk, Coranda, Schmiedeberg and Fr. Walter, Hallervorden, and Pohl and Münzer,[2] on the behavior of ammonium salts in the animal body and the elimination of the ammonia under various conditions, have shown that not only ammonium carbonate, but also those ammonium salts which are burnt into carbonate in the organism, are transformed into urea by carnivora as well as herbivora. v. Schroeder,[3] by irrigating the surviving dog's liver with blood treated with ammonium carbonate or ammonium formate, has shown that the formation of urea takes place, at least in part, in this organ. Nencki, Pawlow, Zaleski and Salaskin[4] have also found that in dogs the quantity of ammonia in the blood from the portal vein is considerably greater than that from the hepatic vein, and they claim that the liver retains in great part the ammonia thus supplied. The formation of urea from ammonia in the liver is a positively proved fact, and the urea formation from ammonium carbonate is to be considered as a synthesis with the elimination of water.

The assumption of a splitting off of ammonia from amino-acids is not difficult of conception, as now, especially from the investigations mentioned in Chapter VIII, we know with positiveness that deamidation of amino-acids does take place in the animal body. The ammonia split off finds in the blood and tissues the carbon dioxide necessary for the formation of carbonate, and to all appearances the conditions are also suitable for the formation of carbamate.

Important observations have been made which give support to the views of Schultzen and Nencki,[5] namely, that the amino-acids are transformed into urea with carbamic acid as an intermediate step. **Drechsel** has shown that the amino-acids yield carbamic acid by oxidation in alkaline fluid outside of the organism, and he obtained urea from ammonium carbamate by passing an alternating electric current through its solution, i.e., by alternate oxidation and reduction. **Drechsel** has also been able to detect small quantities of carbamates in blood, and later in conjunction with **Abel** he detected carbamic acid in alkaline horse's urine. **Drechsel** therefore accepts the formation of urea from ammonium carbamate, and according to him the alternating oxidation and reduction take place in the following way:

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[1] Zeitschr. f. physiol. Chem., 47.

[2] v. Knieriem, Zeitschr. f. Biologie, 10; Feder, *ibid.*, 13; Salkowski, Zeitschr. f. Biologie, 1; Munk, *ibid.*, 2; Coranda, Arch. f. exp. Path. u. Pharm., 12; Schmiedeberg and Walter, *ibid.*, 7; Hallervorden, *ibid.*, 10; Pohl and Münzer, Arch. f. exp. Path. u. Pharm., 43.

[3] Arch. f. exp. Path. u. Pharm., 15. See also Salomon, Virchow's Arch., 97.

[4] Arch. des sciences biol. de St. Pétersbourg, 4; see also Chapter VI, p. 241.

[5] Zeitschr. f. Biologie, 8.

$$\underset{\text{Ammonium carbamate}}{H_4N.O.CO.NH_2}+O=H_2N.O.CO.NH_2+H_2O$$

$$H_2N.O.CO.NH_2+H_2=\underset{\text{Urea}}{H_2N.CO.NH_2}+H_2O.$$

wrong

Abel and **Muirhead**[1] have later observed an abundant elimination of carbamic acid in human and dog's urine after the administration of large quantities of milk of lime, and the probability of the regular appearance of this acid in normal acid-reacting human and dog's urine has been demonstrated by M. **Nencki** and **Hahn**.[2] These last-mentioned investigators have also given very important support to the theory of the formation of urea from ammonium carbamate by observations on dogs with **Eck**'s fistula. In this case the portal vein is directly connected with the inferior vena cava, and communication is thus established between the two, so that the blood of the portal vein flows directly into the vena cava, without passing through the liver. **Nencki** and **Hahn** observed violent symptoms of poisoning in dogs fed on meat and operated upon by **Pawlow** and **Massen**, and these symptoms were quite identical with those obtained on introducing carbamate into the blood.[3] These symptoms also appear after the introduction of carbamate into the stomach, while the introduction of carbamate into the stomach of a normal dog had no action. As these observers also found that the urine of the dog on which the operation was made was richer in carbamate than that of the normal dog, they concluded that the symptoms were due to the non-transformation of the ammonium carbamate into urea in the liver, and they consider the ammonium carbamate as the substance from which the urea is derived in the mammalian liver.

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[1] Drechsel, Ber. d. sächs. Gesellsch. d. Wissensch., 1875. See also Journ. f. prakt. Chem. (N. F.), **12**, **16**, and **22**; Abel, Arch. f (Anat. u.) Physiol., 1891; Abel and Muirhead, Arch. f. exp. Path. u. Pharm., **31**.

[2] Hahn, Massen, Nencki et Pawlow, La fistule d'Eck de la veine cave inférieure et de la veine porte, etc. Arch. des sciences biol. de St. Pétersbourg, **1**, No. 4, 1892.

[3] Rothberger and Winterberg, Zeitschr f. exp. Path. u. Therap., **1**, have found that the phenomena of meat-poisoning and the carbamic-acid intoxication are not identical.

The view as to the formation of urea from ammonium carbamate does not contradict the above statement as to the transformation of the carbonate into urea, since we can imagine that the carbonate is first converted into carbamate with the expulsion of a molecule of water, and that this then is transformed into urea with the expulsion of a second molecule of water.

F. HOFMEISTER[1] has found in the oxidation of different members of the fat series, as well as in amino-acids and proteins, that urea was formed in the presence of ammonia, and he therefore suggests the possibility that urea may be formed by an oxidation-synthesis. According to him, in the oxidation of nitrogenous substances a radical $CONH_2$, containing the amide group, unites at the moment of formation with the radical NH_2 remaining on the oxidation of ammonia, forming urea.

Besides the above-mentioned theories as to the formation of urea, there are others which will not be given, because the only theory which has thus far been positively demonstrated is the formation of urea from ammonium compounds and amino-acids in the liver.

The liver is the only organ in which, up to the present time, a formation of urea has been directly detected;[2] and the question arises, what importance has this urea formation which takes place in the liver? Is the urea wholly or chiefly formed in the liver?

If the liver is the only organ capable of forming urea, it is to be expected, on the extirpation or atrophy of that organ, that a reduced or, in short experiments, at least a strongly diminished elimination of urea should occur. As at least a part of the urea is formed in the liver from ammonium compounds, a simultaneous increase in the elimination of ammonia is to be expected.

The extirpation and atrophy experiments made on animals by different, methods by NENCKI and HAHN, SLOSSE, LIEBLEIN, NENCKI and PAWLOW SALASKIN and ZALESKI[3] have shown that sometimes a rather marked increase of ammonia and a diminished elimination of urea takes place after the operation, but also that there are cases in which, irrespective of the pronounced atrophy, an abundant formation of urea occurs, and no appreciable, if any, change in the proportion of ammonia to the total nitrogen and urea is observed. After shutting out the organs of the posterior part of the body, especially the liver and kidneys, from the circulation, **Kaufmann**[1] also found an important increase in the urea of the blood, and these different observations show that the liver is not the only organ, in the various animals experimented upon, in which urea is formed.

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[1] Arch. f. exp. Path. u. Pharm., 37.

[2] In regard to the investigations of Prevost and Dumas, Meissner, Voit, Gréhant, Gscheidlen and Salkowski, and others, on the rôle of the kidneys in the formation of urea, see v. Schroeder, Arch. f exp. Path. u. Pharm., 15 and 19, and Voit, Zeitschr. f. Biologie, 4.

[3] Nencki and Hahn, l. c.; Slosse, Arch. f. (Anat. u.) Physiol., 1890; Lieblein, Arch. f. exp. Path. u. Pharm., 33; Nencki and Pawlow, Arch. des scienc. biol. de St. Pétersbourg, 5. See also v. Meister, Maly's Jahresbr., 25; Salaskin and Zaleski, Zeitschr. f. physiol. Chem., 29.

The observations made by numerous investigators[2] on human beings with cirrhosis of the liver, acute yellow atrophy of the liver, and phosphorus poisoning have led to the same result. These investigations teach that in certain cases the proportion of the nitrogenous substances may be so changed that urea is only 50–60 per cent of the total nitrogen, while in other cases, on the contrary, even in very extensive atrophy of the liver-cells, the formation of urea is not diminished, neither is the proportion between the total nitrogen, urea, and ammonia essentially changed. Even in the cases in which the formation of urea was relatively diminished and the elimination of ammonia considerably increased further investigation must be instituted before it will be possible to assume a reduced ability of the organism to produce urea. An increased elimination of ammonia may, as shown by **Münzer** in the case of acute phosphorus poisoning, be dependent upon the formation of abnormally large quantities of acids, caused by abnormal metabolism, and these acids require a greater quantity of ammonia for their neutralization according to the law of elimination of ammonia, which will be given later. That an abnormal formation of acid occurs after the cutting out of the liver has been especially shown by **Salaskin** and **Zaleski**.[3]

For the present we are not justified in the statement that the liver is the only organ in which urea is formed, and only continued investigation can yield further information as to the extent and importance of the formation of urea in the liver from ammonium compounds.

Properties and Reactions of Urea. Urea crystallizes in needles or in long, colorless, four-sided, often hollow, anhydrous rhombic prisms. It has a neutral reaction, and produces a cooling sensation on the tongue like saltpeter. It melts at 132° C. At ordinary temperatures it dissolves in an equal weight of water and in five parts alcohol; it requires one part boiling alcohol for solution; it is insoluble in alcohol-free anhydrous ether, and also in chloroform. If urea in substance is heated in a test-tube, it melts, decomposes, gives off ammonia, and leaves finally a non-transparent white residue which, among other substances, contains cyanuric acid and *biuret*, which latter dissolves in water, giving a beautiful reddish-violet liquid with copper sulphate and alkali (*biuret reaction*). On heating with baryta-water or caustic alkali, also in the so-called alkaline fermentation of urine caused by micro-organisms, urea splits into carbon dioxide and ammonia with the addition of water. The same decomposition products are produced when urea is heated with concentrated sulphuric acid. An alkaline solution of sodium hypobromite decomposes urea into nitrogen, carbon dioxide, and water according to the equation

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[1] Compt. rend. soc. biol., **46**, and Arch. de Physiol. (5), **6**.

[2] See Hallervorden, Arch. f. exp. Path. u. Pharm., **12**; Weintraud, *ibid.*, **31**; Münzer and Winterberg, *ibid.*, **33**; Stadelmann, Deutsch. Arch. f. klin. Med., **33**; Fawitzki, *ibid.*, **45**; Münzer, *ibid.*, **52**; Fränkel, Berlin. klin. Wochenschr., 1878; Richter, *ibid.*, 1896; Mörner and Sjöqvist, Skand. Arch. f. Physiol., **2**, and Sjöqvist, Nord. Med. Arkiv, 1892; Gumlich, Zeitschr. f. physiol. Chem., **17**; v. Noorden, Lehrb. d. Pathol. des Stoffwechsels, 2. Aufl., Bd. 1, 104.

[3] Zeitschr. f. physiol. Chem., **29**.

$$CON_2H_4 + 3NaOBr = 3NaBr + CO_2 + 2H_2O + N_2.$$

With a concentrated solution of furfurol and hydrochloric acid urea in substance gives a coloration passing from yellow, green, blue, to violet, and then beautiful purple-violet after a few minutes (SCHIFF's reaction). According to HUPPERT[1] the test is best performed by taking 2 c.c. of a concentrated furfurol solution, 4–6 drops of concentrated hydrochloric acid, and adding to this mixture, which must not be red, a small crystal of urea. A deep violet coloration appears in a few minutes.

Urea forms crystalline compounds with many acids. Among these the one with nitric acid and the one with oxalic acid are the most important.

UREA NITRATE, $CO(NH_2)_2.HNO_3$. On crystallizing quickly this compound forms thin rhombic or six-sided overlapping tiles, or colorless plates, with an angle of 82°. When crystallizing slowly, larger and thicker rhombic pillars or plates are obtained. This compound is rather easily soluble in pure water, but is considerably less soluble in water containing nitric acid; it may be obtained by treating a concentrated solution of urea with an excess of strong nitric acid free from nitrous acid. On heating this compound it volatilizes without leaving a residue.

This compound may be employed with advantage in detecting small amounts of urea. A drop of the concentrated solution is placed on a microscope-slide and the cover-glass placed upon it; a drop of nitric acid is then placed on the side of the cover-glass and allowed to flow under. The formation of crystals begins where the solution and the nitric acid meet. Alkali nitrates may crystallize very similarly to urea nitrate when they are contaminated with other bodies: therefore, in testing for urea, the crystals must be identified as urea nitrate by heating and by other means.

UREA OXALATE, $2.CO(NH_2)_2.H_2C_2O_4$. This compound is more sparingly soluble in water than the nitric-acid compound. It is obtained in rhombic or six-sided prisms or plates on adding a saturated oxalic-acid solution to a concentrated solution of urea.

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[1] Huppert-Neubauer, Analyse des Harns, 10. Aufl., 296.

Urea also forms combinations with mercuric nitrate in variable proportions. If a very faintly acid mercuric-nitrate solution is added to a 2 per cent solution of urea and the mixture carefully neutralized, a compound is obtained of a constant composition which contains for every 10 parts of urea 72 parts of mercuric oxide. This compound serves as the basis of LIEBIG's titration method. Urea combines also with salts, forming mostly crystallizable combinations, as, for instance, with sodium chloride, with the chlorides of the heavy metals, etc. An alkaline but not a neutral solution of urea is precipitated with mercuric chloride.

If urea is dissolved in dilute hydrochloric acid and then an excess of formaldehyde is added, a thick, white, granular precipitate is obtained which is difficultly soluble and whose composition is somewhat disputed.[1] With phenylhydrazine, urea in strong acetic acid gives a colorless crystalline compound of phenylsemicarbazid, $C_6H_5NH.NH.CONH_2$, which is soluble with difficulty in cold water and melts at 172° C. (JAFFÉ [2]).

The method of preparing urea from urine is in the main as follows: Concentrate the urine, which has been faintly acidified with sulphuric acid, at a low temperature, add an excess of nitric acid, at the same time keeping the mixture cool, press the precipitate well, decompose it in water with freshly precipitated barium carbonate, dry on the water-bath, extract the residue with strong alcohol, decolorize when necessary with animal charcoal, and filter while warm. The urea which crystallizes on cooling is purified by recrystallization from warm alcohol. A further quantity of urea may be obtained from the mother-liquor by concentration. The urea is purified from contaminating mineral bodies by redissolving in alcohol-ether. If it is only necessary to detect the presence of urea in urine, it is sufficient to concentrate a little of the urine on a watch-glass and, after cooling, treat it with an excess of nitric acid. In this way we obtain crystals of urea nitrate.

Quantitative Estimation of the Total Nitrogen and Urea in Urine. Among the various methods proposed for the estimation of the total nitrogen, that suggested by KJELDAHL is to be recommended. But as LIEBIG's method for the estimation of urea is really a method for determining the total nitrogen, and as the physician has not always at hand the apparatus and utensils necessary for a KJELDAHL determination, he often makes use of this method; hence both will be given in detail.

KJELDAHL's method consists in transforming all the nitrogen of the organic substances into ammonia by heating with a sufficiently concentrated sulphuric acid. The ammonia is distilled off after supersaturating with alkali and the ammonia collected in standard sulphuric acid. The following reagents are necessary:

1. *Sulphuric Acid.* Either a mixture of equal volumes of pure concentrated and fuming sulphuric acid or else a solution of 200 grams phosphoric

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[1] See Tollens and his pupils, Ber. d. deutsch. chem. Gesellsch., **29**, 2751; Goldschmidt, *ibid.*, **29**, and Chem. Centralbl., 1897, **1**, 33; Thoms, *ibid.*, **2**, 144 and 737.

[2] Zeitschr. f. physiol. Chem., **22**.

anhydride in 1 liter of pure concentrated sulphuric acid. 2. *Caustic soda* free from nitrates, 30–40 per cent solution. The quantity of this caustic-soda solution necessary to neutralize 10 c.c. of the acid mixture must be determined. 3. Metallic *mercury* or pure yellow *mercuric oxide*. (The addition of this facilitates the destruction of the organic substances.) 4. A *potassium-sulphide* solution of 4 per cent, whose object is to decompose any mercuric amide combination which might not evolve its ammonia completely during the distillation with caustic soda. 5. N/5 sulphuric acid and N/5 caustic-soda solution.

In performing the determination 5 c.c. of the carefully measured and filtered urine is placed in a long-neck KJELDAHL flask, a drop of mercury or about 0.3 gram of mercuric oxide added, and then treated with 10–15 c.c. of the strong sulphuric acid. The contents are heated very carefully, placing the flask at an angle, until they just begin to boil gently; this is continued for about half an hour after the mixture becomes colorless. On cooling the contents are transferred to a voluminous distilling-flask, carefully washing the KJELDAHL flask with water, and the greater part of the acid is neutralized by caustic soda. A few zinc shavings are added to prevent too rapid ebullition on distillation, and then an excess of caustic-soda solution which has previously been treated with 30–40 c.c. of the potassium-sulphide solution. The flask is quickly connected with the condenser-tube and all the ammonia distilled off. In order to prevent loss of ammonia it is best to lower the end of the exit-tube below the surface of the acid, and the regurgitation of the acid is prevented by having a bulb blown on the exit-tube. Not less than 25–30 c.c. of the standard acid is used for every 5 c.c. of urine, and on completion of the distillation the acid is retitrated with N/5 caustic soda, using rosolic acid, tincture of cochineal, or lacmoid as indicator. Each cubic centimeter of the acid corresponds to 2.8 milligrams nitrogen. As a control and in order to test the purity of the reagents, or to eliminate any error caused by an accidental quantity of ammonia in the air, we always make a blank determination with the reagents.

LIEBIG'S METHOD is based upon the fact that a dilute solution of mercuric nitrate under proper conditions precipitates all the urea from its solution, forming a compound of constant composition. As indicator, a soda solution or a thin paste of sodium bicarbonate is used. An excess of mercuric nitrate produces herewith a yellow or yellowish-brown compound, while the compound of urea and mercury is white. PFLÜGER[1] has given full particulars for this method; therefore we will describe PFLÜGER'S modification of LIEBIG'S method.

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[1] Pflüger, and Pflüger and Bohland, in Pflüger's Arch., **21, 36, 37, and 40.**

As phosphoric acid is also precipitated by the mercuric-nitrate solution, this must be removed from the urine by the addition of a baryta solution before titration. PFLÜGER also suggested that the acidity produced by the mercury solution be neutralized during titration by the addition of a soda solution. The liquids necessary for the titration are the following:

1. *Mercuric-nitrate Solution.* This solution is calculated for a 2 per cent urea solution, and 20 c.c. of the first should correspond to 10 c.c. of the latter. Each cubic centimeter of the mercury solution corresponds to 0.01 gram urea. As a small excess of HgO is necessary in the urine to cause the final reaction (with alkali carbonate or bicarbonate) to appear, each cubic centimeter of the mercury solution must contain 0.0772 instead of 0.0720 gram HgO. The mercury solution contains therefore 77.2 grams HgO in 1 liter.

The solution may be prepared from pure mercury or mercuric oxide by dissolving in nitric acid. The solution, freed as completely as possible from an excess of acid, is diluted by the careful addition of water, stirring meanwhile, until it has a specific gravity of 1.10, or a little higher, at 20° C. The solution is standardized with a 2 per cent solution of pure urea which has been dried over sulphuric acid and the operation conducted as will be described later. If the solution is too concentrated, it is corrected by carefully adding the necessary amount of water, avoiding the precipitation of the basic salt, and titrating again. The solution is correct if 19.8 c.c. of it, added at once to 10 c.c of the urea solution and the quantity (11–12 c.c. or more) of normal soda solution necessary to nearly completely neutralize the liquid, gives the final reaction when exactly 20 c.c. of the mercury solution has been employed.

2. *Baryta Solution.* This consists of 1 vol. of barium nitrate and 2 vols. of barium-hydrate solution, both saturated at the ordinary temperature.

3. *Normal Soda Solution.* This solution contains 53 grams of pure anhydrous sodium carbonate in 1 liter of water. According to PFLÜGER a solution having a specific gravity of 1.053 is sufficient. The amount of this soda solution necessary to completely neutralize the acid set free during the titration is determined by titrating with a pure 2 per cent urea solution. To facilitate operations a table can be made showing the quantity of soda solution necessary when from 10 to 35 c.c. of the mercury solution is used.

Before the titration the following must be considered. The chlorides of the urine interfere with the titration in that a part of the mercuric nitrate is transformed into mercuric chloride, which does not precipitate the urea. The chlorides of the urine are therefore removed by a silver-nitrate solution, which also removes any bromine or iodine compounds which may exist in the urine. If the urine contains proteid in noticeable amounts, it must be removed by coagulation and the addition of acetic acid, but care must be taken that the concentration and the volume of the urine are not changed during these operations. If the urine contains ammonium carbonate in noticeable quantities, caused by alkaline fermentation, this titration method cannot be applied. The same is true of urine containing leucine, tyrosine, or medicinal preparations precipitated by mercuric nitrate.

In cases where the urine is free from proteid er sugar and not specially poor in chlorides, the quantity of urea, and also the approximate quantity of mercuric nitrate necessary for the titration, may be learned from the specific gravity. A specific gravity of 1.010 corresponds to about 10 p. m., a specific gravity of 1.015 generally somewhat less than 15 p. m., and a specific gravity of 1.015–1.020 about 15–20 p. m. urea. With a specific gravity higher than 1.020 the urine generally contains more than 20 p. m. of urea, and above this point the amount of urea increases much more rapidly than the specific gravity, so that with a specific gravity of 1.030 it contains over 40 p. m. urea. Fever-urines with a specific gravity above 1.020 sometimes contain 30–40 p. m. urea, or even more.

PREPARATION FOR THE TITRATION. If a large amount of urea is suspected from a high specific gravity, the urine must first be diluted with a carefully measured quantity of water, so that the amount of urea is reduced below 30 p. m. In a special portion of the same urine the amount of chlorides is determined.by one of the methods which will be given later, and the number of cubic centimeters of silver-nitrate solution necessary is noted. Then a larger quantity of urine, say 100 c.c., is mixed with one-half or, if this is not sufficient to precipitate all the sulphuric and phosphoric acids, with an equal volume of the baryta solution; it is then allowed to stand a little while, and the precipitate is filtered through a dried filter. From the filtrate containing the urine diluted with water a proper quantity, corresponding to about 60 c.c. of the original urine, is measured, and exactly neutralized with nitric acid added from a burette, so that the exact quantity employed is known. The neutralized mixture of urine and baryta is treated with the proper quantity of silver-nitrate solution necessary to completely precipitate the chlorides, which were ascertained by a previous determination. The mixture, containing a known volume of urine, is now filtered through a dried filter into a flask, and from the filtrate an amount is measured off corresponding to 10 c.c. of the original urine.

EXECUTION OF THE TITRATION. Nearly the whole quantity of the mercuric-nitrate solution, which is judged from the specific gravity of the urine to be the minimum amount required, is added at once, and immediately afterwards the quantity of soda solution necessary, as indicated by the table. If the mixture becomes yellowish in color, then too much mercury solution has been added and another determination must be made. If the test remains white, and if a drop taken out and placed on a glass plate with a dark background and stirred with a drop of a thin paste of sodium bicarbonate does not give a yellow color, the addition of mercury solution is continued by adding repeatedly at first $\frac{1}{2}$ and later $\frac{1}{10}$ c.c., and testing after each addition in the following way: A drop of the mixture is placed on a glass plate with a dark background beside a small drop of the bicarbonate paste. If the color after stirring the two drops together is still white after a few seconds, then more mercury solution must be added; if, on the contrary, it is yellowish, then—if not too much mercury solution has been added by inattention—the result to $\frac{1}{10}$ c.c. has been found. By this approximate determinatio, which is sufficient in many cases, we haven fixed the minimum amount of mercury solution necessary to add to the quantity of urine in question, and we now proceed to the final determination.

A second quantity of the filtrate, corresponding to 10 c.c. of the original urine, is filtered, and the same quantity of mercury solution added at one time as was found necessary to produce the final reaction, and immediately after the corresponding amount of soda solution, which must not indicate the end of the reaction. Then continue adding the mercury solution $\frac{1}{10}$ c.c. at a time without neutralizing with soda, until a drop taken out and mixed with the soda solution gives a yellow coloration. If this final reaction is obtained after the addition of 0.1–0.2 c.c., then the titration may be considered as finished. If, on the contrary, a larger quantity is necessary, the addition of the mercury solution must be continued until a final reaction is obtained with simple carbonate, and the titration repeated again, adding the quantity of mercury solution used in the previous test at one time, and also adding the corresponding amount of soda solution. If then the end reaction is obtained by the addition of $\frac{1}{10}$ c.c., the titration may be considered as finished.

If in each titration a quantity of filtrate containing urine and baryta corresponding to 10 c.c. of the original urine is used, then the calculations are very simple, since 1 c.c. of mercuric-nitrate solution corresponds to 0.01 gram of urea. As the mercury solution is made for a 2 per cent urea solution, and as the filtrate of urine and baryta generally contains less urea (if the quantity of urea is above 2 per cent, it is easy to avoid any mistake by diluting the urine at the beginning of the operation), a mistake occurs here which can be corrected in the following way, according to PFLÜGER: To the measured volume of the filtrate from the urine (the filtrate with baryta after neutralization with nitric acid, precipitation with silver nitrate and filtration) the quantity of normal soda solution employed is added, and from this sum the volume of mercury solution used is subtracted. The remainder is then multiplied by 0.08, and the product subtracted from the number of cubic centimeters of mercury solution used. For example, if the filtrate (urine and baryta + nitric acid + silver nitrate) measured 25.8 c.c., and the number of cubic centimeters of soda solution used in the titration was 13.8 c.c., and of the mercury solution 20.5 c.c., we have then $20.5-[(39.6-20.5)\times 0.08]=20.5-1.53=18.97$, and the corrected quantity of mercury solution is therefore 18.97 c.c. If the cubic centimeters of the filtrate (in this case 25.8 c.c.) correspond to 10 c.c. of the original urine, then the amount of urea is $18.97\times 0.01=0.1897=18.97$ p. m. urea.

Besides the urea other nitrogenous constituents of the urine are precipitated by the mercury solution. In the titration we really do not obtain the quantity of urea, but, as PFLÜGER has shown, the total quantity of nitrogen in the urine expressed as urea. As urea contains 46.67 per cent N, the total quantity of nitrogen in the urine may be calculated from the quantity of urea found. The results obtained by this calculation correspond well, according to PFLÜGER, with the results found for the total nitrogen as determined by KJELDAHL's method.

GLASSMANN[1] has recently suggested a modification of the LIEBIG-PFLÜGER titration method which consists in precipitating the urea with an excess of mercuric-nitrate solution and then determining the excess of mercuric nitrate in the filtrate by means of ammonium sulphocyanide.

Among the methods suggested for the special estimation of urea, that of MÖRNER-SJÖQVIST, in combination with FOLIN's method, is perhaps the most trustworthy and readily performed. For this reason only this method will be given in detail, while we must refer to special works for the other methods, such as BUNSEN's method with its many modifications as suggested by PFLÜGER, BOHLAND and BLEIBTREU.[2]

Principle of Mörner-Sjöqvist's Method.[3] According to this method the nitrogenous constituents of the urine, with the exception of urea, ammonia, hippuric acid, creatinine, and traces of allantoin, are precipitated by a mixture of alcohol and ether after the addition of a solution of barium chloride and barium hydrate or in the presence of sugar with solid barium hydrate. The urea is determined in the concentrated filtrate, after driving off the ammonia, by KJELDAHL's nitrogen estimation. Because of the slight error due to the presence of hippuric acid and creatinine, several modifications have been suggested by SALASKIN and ZALESKI and by BRAUNSTEIN.[4] These errors are best prevented, according to MÖRNER, by the use of FOLIN's method.

Principle of Folin's Method.[5] On heating urea with hydrochloric acid and crystalline magnesium chloride, which melts in its water of crystallization at 112–115° C. and then boils at about 150–155° C., the urea is completely decomposed, while no appreciable decomposition of the hippuric acid and creatinine takes place. The ammonia produced from the urea is distilled off and determined by titration. The amount of ammonia previously existing in the urine must be specially determined.

Determination of Urea by the Mörner-Sjöqvist and Folin Method.[6] Five c.c. of the urine are treated with 1.5 grams of powdered barium hydroxide, and when as much of this is dissolved as possible by gently mixing, it is precipitated by 100 c.c. of the alcohol and ether mixture (⅓ vol. ether). On the following day it is filtered and the precipitate washed with the alcohol and ether mixture. The alcohol and ether are distilled off from the filtrate at about 55° C. (not above 60° C.). The remaining liquid is treated with 2 c.c. of hydrochloric acid of sp. gr. 1.124 (for 5 c.c. urine), and carefully transferred to a flask of 200 c.c. capacity, and evaporated to dryness on the water-bath. Then add 20 grams of crystalline magnesium chloride to the contents of the flask and 2 c.c. of concentrated hydrochloric acid, and boil on a wire gauze over a small flame for two hours, making use of a proper return cooler. After cooling it is diluted to about ¾ to 1 liter with water, the ammonia completely distilled off after making it alkaline with caustic soda, and the ammonia collected in standard acid. After boiling in order to drive off the CO_2 and cooling, the acid is retitrated. Corrections must be made for the ammonia of the urine and for that contained in the magnesium chloride.

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[1] Ber. d. d. chem. Gesellsch., **39**.

[2] Pflüger's Arch., **38**, **43**, and **44**.

[3] Skand. Arch. f. Physiol., **2**, and Mörner, *ibid.*, **14**, where the recent literature may also be found.

[4] Braunstein, Zeitschr. f. physiol. Chem., **31**; Salaskin and Zaleski, *ibid.*, **28**.

[5] *Ibid.*, **32**, **36**, and **37**.

[6] See Mörner, Skand. Arch. f. Physiol., **14**.

If a special determination of the preformed ammonia has been made, then a direct treatment of the urine according to Folin (nevertheless after the evaporation of the urine with hydrochloric acid) gives good results. In the presence of sugar the treatment of the urine with barium hydroxide is absolutely necessary according to Mörner, otherwise the humin substances produced from the sugar take up and retain nitrogen.

Knop-Hüfner's method[1] is based on the fact that urea, by the action of sodium hypobromite, splits into water, carbon dioxide (which dissolves in the alkali), and nitrogen, whose volume is measured (see page 555). This method is less accurate than the preceding ones, and therefore in scientific work it is discarded. It is of value to the physician and for practical purposes, because of the ease and rapidity with which it may be performed, even though it may not give very accurate results. For practical purposes a number of different apparatuses have been constructed to facilitate the use of this method.

For the quantitative estimation of urea in blood or other animal fluids, as well as in the tissues, Schöndorff has proposed a method where the proteins and extractives are first precipitated by a mixture of phosphotungstic acid and hydrochloric acid, and then the filtrate made alkaline with lime. The quantity of ammonia formed on heating a part of this filtrate to 150° C. with phosphoric acid and the amount of carbon dioxide produced by heating the other part to 150° C. are determined. In regard to the principles of this method, as well as to the details, we refer to the original article (Pflüger's Arch., 62). See also Hoppe-Seyler-Thierfelder's Handbuch, 7. Aufl.

Urein is the name given by Ovid Moor to a product which he obtained by extracting urine, which had been evaporated to a syrup, with absolute alcohol and precipitating the urea with alcohol containing oxalic acid, or by cooling and treatment with alcohol. Urein is a golden-yellow oil which is poisonous; it reduces permanganate in the cold, and it forms the chief portion of the nitrogenous extractives of urine. There is no doubt but that urein is a mixture of substances. According to Moor,[2] the amount of urea in the urine is only about one-half that ordinarily given, and he has suggested a new method for the determination of the true quantity of urea. The possibility that in the urine we have other bodies besides urea which have been determined with the urea cannot be denied *a priori*. From the investigations published so far it must be said that MOOR's assertions are not sufficiently grounded.[1]

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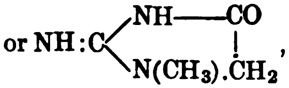
[1] Knop, Zeitschr. f. analyt. Chem., **9**; Hüfner, Journ. f. prakt. Chem. (N. F.), **3**. In regard to the extensive literature, see Huppert-Neubauer, 10. Aufl., 304, and following.

[2] O. Moor, Bull. Acad. de St. Pétersbourg, **14** (also Maly's Jahresber., **31**, **415**), and Zeitschr. f. Biologie, **44** and **45**, and Zeitschr. f. physiol. Chem., **40**.

Carbamic Acid, $CH_3NO_2 = CO < {NH_2 \atop OH}$. This acid is not known in the free state, but only as salts. Ammonium carbamate is produced by the action of dry ammonia on dry carbon dioxide. Carbamic acid is also produced by the action of potassium permanganate on protein and several other nitrogenous organic bodies.

The occcurrence of carbamic acid in human and animal urines has already been considered in connection with the formation of urea. The calcium salt, which is soluble in water and ammonia but insoluble in alcohol, is the most important in the detection of this acid. The solution of the calcium salt in water becomes cloudy on standing, but much more quickly on boiling, and calcium carbonate separates. NOLF, MACLEOD and HASKINS have made experiments as to the method of formation of carbamic acid. The latter have indicated a new method for the quantitative estimation of carbamates.[2]

Carbamic-acid ethylester (urethane), as shown by JAFFE,[3] may pass, by the mutual action of alcohol and urea, into the alcoholic extract of urine when one is working with large quantities.

Creatinine, $C_4H_7N_3O$, or  $NH:C\begin{cases} NH-CO \\ N(CH_3).CH_2 \end{cases}$, is generally considered as the anhydride of creatine (see page 455) found in the muscles. It occurs in human urine and in that of certain mammalia. It has also been found in ox-blood, milk, though in very small amounts, and in the flesh of certain fishes.

JOHNSON's statement that the creatinine of the urine is different from that produced by the action of acids on creatine is incorrect according to TOPPELIUS and POMMEREHNE, WOERNER and THIELEN.[4]

The quantity of creatinine in human urine is, in a grown man voiding a normal quantity of urine in the course of a day, 0.6–1.3 grams (NEUBAUER), or on an average 1 gram. JOHNSON[5] found 1.7–2.1 grams per day, and similar results have been obtained by HOOGENHUYZE and VERPLOEGH.[6] The quantity of creatinine with a diet free from meat is, according to FOLIN,[7] somewhat variable for different individuals, but is constant for the same person. He found the quantity never below 1 gram and often between 1.3 and 1.7 grams. Nurslings also eliminate creatinine, although the quantity is only small (Hoogenhuyze and Verploegh). The quantity of creatinine is dependent upon the food in so far as it is increased by meat diet, but otherwise, according to Folin, it is not dependent upon the food. The creatinine is, according to him, the product of the endogenous metabolism of the cells, and its quantity does not depend, as was also shown later by Klercker, upon the quantity of protein food introduced and catabolized. The elimination of creatinine, therefore, does not run parallel with the elimination of urea and is not correspondingly greater with food very rich in protein than with food very poor in protein.

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[1] See Kubiabko, Maly's Jahresber., **31**, 415; Erben, Zeitschr. f. physiol. Chem. **38**; Folin, *ibid.*, **37**; Gies, Journ. Amer. Chem. Soc., **25**; Haskins, Amer. Journ. of Physiol., **12**; Lippich, Zeitschr. f. physiol. Chem., **48**.

[2] Nolf, Zeitschr. f. physiol. Chem., **23**; Macleod and Haskins, Amer. Journ. of Physiol., **12**.

[3] Zeitschr. f. physiol. Chem., **14**.

[4] S. Johnson, Proceed. Roy. Soc., **42, 43**; Chem. News, **55**; Toppelius and Pommerehne, Arch. f. Pharm., **234**; Woerner, Arch. f. (Anat. u.) Physiol., 1898.

[5] Huppert-Neubauer, Harnanalyse, 10. Aufl., 387.

[6] Zeitschr. f. physiol. Chem., **46**.

[7] Amer. Journ. of Physiol. **13**; af. Klercker, Hofmeister's Beiträge, **8**.

The statements as to the behavior of the creatinine elimination with work are very contradictory.[1] Hoogenhuyze and Verploegh, who made use of a much more trustworthy method of quantitative estimation than their predecessors, find that muscular activity as a rule does not cause any rise in the creatinine elimination, and that in man such a rise with work occurs only when the body is obliged to live upon its own tissues. Little is known about the behavior of creatinine in diseases. In cases with an increase in metabolism the quantity is said to rise, while in other cases, as in anæmia and cachexia with reduced metabolism, the quantity is lessened.

Creatinine crystallizes in colorless, shining monoclinic prisms which differ from creatine crystals in not becoming white with loss of water when heated to 100° C. It dissolves in 11 parts cold water, but more easily in warm water. It is difficultly soluble in cold alcohol, but the statements in regard to its solubilities differ widely.[2] It is more soluble in warm alcohol and nearly insoluble in ether. In alkaline solution creatinine is converted into creatine very easily on warming.

Creatinine gives an easily soluble crystalline compound with hydrochloric acid. A solution of creatinine acidified with mineral acids gives crystalline precipitates with phosphotungstic and phosphomolybdic acids even in very dilute solutions (1:10 000) (Kerner, Hofmeister[3]). It is precipitated, like urea, by mercuric-nitrate solution and also by mercuric chloride. On treating a dilute creatinine solution with sodium acetate and then with mercuric chloride a precipitate of glassy globules having the composition $4(C_4H_7N_3O.HCl.HgO)3HgCl_2$ separates on standing some time (Johnson). Among the compounds of creatinine, that with zinc chloride, *creatinine zinc chloride*, $(C_4H_7N_3O)_2ZnCl_2$, is of special interest. This combination is obtained when a sufficiently concentrated solution of creatinine in alcohol is treated with a concentrated, faintly acid solution of zinc chloride. Free mineral acids dissolve the compound, hence they must not be present; this, however, may be prevented by an addition of sodium acetate. In the impure state, as ordinarily obtained from urine, creatinine zinc chloride forms a sandy, yellowish powder which under the microscope appears as fine needles forming concentric groups, mostly complete rosettes or yellow balls or tufts, or grouped as brushes. On slowly crystallizing or when very pure, more sharply defined prismatic crystals are obtained. The compound is sparingly soluble in water.

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[1] The literature on this subject may be found in Hoogenhuyze and Verploegh, l. c.

[2] See Huppert-Neubauer, 10. Aufl. and Hoppe-Seyler-Thierfelder's Handbuch, 7. Aufl.

[3] Kerner, Pflüger's Arch., 2; Hofmeister, Zeitschr. f. physiol. Chem., 5.

Creatinine acts as a reducing agent. Mercuric oxide is reduced to metallic mercury, and oxalic acid and methylguanidine (methyluramine) are formed. Creatinine also reduces cupric hydrate in alkaline solution, forming a colorless soluble compound, and only after continued boiling with an excess of copper salt is free subxoide of copper formed. Creatinine interferes with TROMMER's test for sugar, partly because it has a reducing action and partly by retaining the copper suboxide in solution. The compound with copper suboxide is not soluble in a saturated soda solution, and if a little creatinine is dissolved in a cold saturated soda solution and then a few drops of FEHLING's reagent added a white flocculent compound separates after heating to 50–60° C. and then cooling (v. MASCHKE's[1] reaction). An alkaline bismuth solution (see Sugar Tests) is not reduced by creatinine.

If we add a few drops of a freshly prepared very dilute sodium-nitroprusside solution (sp. gr. 1.003) to a dilute creatinine solution (or to the urine) and then a few drops of caustic soda, a ruby-red liquid is obtained which quickly turns yellow again (WEYL's[2] reaction). If the cold yellow solution is neutralized and treated with an excess of acetic acid a crystalline precipitate of a nitroso-compound ($C_4H_6N_4O_2$) of creatinine separates on stirring (KRAMM[3]). If, on the contrary, the yellow solution is treated with an excess of acetic acid and heated, the solution becomes first green and then blue (SALKOWSKI[4]); finally a precipitate of Prussian blue is obtained. If a solution of creatinine in water (or urine) is treated with a watery solution of picric acid and a few drops of a dilute caustic-soda solution, a red coloration lasting several hours occurs immediately at the ordinary temperature, which turns yellow on the addition of acid (JAFFÉ's[5] reaction). Acetone gives a more reddish-yellow color. Dextrose gives with this reagent a red coloration only after heating.

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[1] Zeitschr. f. analyt. Chem., **17**.

[2] Ber. d. deutsch. chem. Gesellsch., **11**.

[3] Centralbl. f. d. med. Wissensch., **1897**.

[4] Zeitschr. f. physiol. Chem., **4**.

[5] *Ibid.*, **10**.

In preparing creatinine from urine the creatinine zinc chloride is first prepared according to NEUBAUER'S[1] method. One liter or more of urine is treated with milk of lime until alkaline and then $CaCl_2$ solution is added until all the phosphoric acid is precipitated. The filtrate is evaporated to a syrup after faintly acidifying with acetic acid and this is treated while still warm with 97 per cent alcohol (about 200 c.c. for each liter of urine). After about twelve hours it is filtered and the filtrate treated first with a little sodium acetate and then with an acid-free zinc chloride solution of a specific gravity of 1.20 (about 2 c.c. for each liter of urine.) After thorough stirring it is allowed to stand forty-eight hours and the precipitate is collected on a filter and washed with alcohol. The creatinine zinc chloride is dissolved in hot water, boiled with lead oxide, filtered, the filtrate decolorized by animal charcoal, evaporated to dryness, and the residue extracted with strong alcohol (which leaves the creatinine undissolved). The alcoholic extract is evaporated to the point of crystallization, and the crystals purified by recrystallization from water.

Creatinine may also be prepared from urine by precipitating with a mercuric-chloride solution according to either MALY'S or JOHNSON'S[2] process.

The best method for preparing creatinine is the following, suggested by FOLIN.[3] The creatinine is first precipitated as the double picrate of creatinine and potassium by means of picric acid according to JAFFÉ'S method, and then this precipitate, while still moist, is decomposed by $KHCO_3$ and water. The solution, which contains the creatinine besides potassium carbonate and small amounts of impurities, is neutralized with sulphuric acid and the sulphate precipitated by alcohol. The creatinine is now converted into the double zinc-chloride salt and this last treated with moist lead hydroxide. After the removal of the lead the solution contains a mixture of creatinine and creatine, which last is completely transformed into creatinine by heating for 48 hours with normal sulphuric acid. After exact neutralization with barium-hydrate solution it is concentrated to the point of crystallization.

The *quantitative estimation of creatinine* may be performed according to NEUBAUER'S method for the preparation of creatinine, or more simply by SALKOWSKI'S[4] modification of this method. 240 c.c. of the urine freed from proteid (by boiling with acid) and from sugar (by fermentation with yeast) are made alkaline with milk of lime, and precipitated by $CaCl_2$ and made up to 300 c.c.: 250 c.c. (=200 c.c. urine) of this are measured off, neutralized or made only faintly acid with acetic acid and evaporated to about 20 c.c., then thoroughly stirred with an equal volume of absolute alcohol, and completely transferred to a 100, c.c. flask which contains some alcohol, the residue in the dish being washed with alcohol. On thorough shaking and cooling, the flask is filled up to the 100-c.c. mark with absolute alcohol and allowed to stand twenty-four hours. 80 c.c. (=160 c.c. urine) of the filtrate are collected in a beaker and treated with 0.5–1 c.c. of zinc-chloride solution, and the covered beaker is left standing in a cool place for two or three days. The precipitate is collected on a small dried and weighed filter, using the filtrate to wash the crystals from the beaker. After allowing the crystals to completely drain off, they are washed with a little alcohol until the filtrate gives no reaction for chlorine, and dried at 100° C. 100 parts of creatinine zinc chloride contain 62.44 parts of creatinine. As the precipitate is never quite pure, the quantity of zinc must be carefully determined, in exact experiments, by evaporating with nitric acid, heating, washing the oxide of zinc with water (to remove any NaCl), drying, heating, and weighing. 22.4 parts zinc oxide correspond to 100 parts creatinine zinc-chloride. Instead of weighing, the nitrogen can be determined by Kjeldahl's method and the creatinine calculated from this.

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[1] Ann. d. Chem. u. Pharm., **119**.

[2] Maly, Annal. d. Chem. u. Pharm., **159**; Johnson, Proceed. Roy. Soc., **43**.

[3] Zeitschr. f. physiol. Chem., **41**.

[4] Zeitschr. f. physiol. Chem., **10** and **14**.

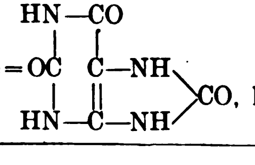
Folin[1] has suggested a colorimetric method for determining creatinine which is based upon Jaffé's picric-acid reaction and is as follows: 10 c.c. of the urine are treated in a graduated flask of 500 c.c. capacity with 15 c.c. of a 1.2 per cent solution of picric acid and 5 c.c. of a 10 per cent NaOH solution. After shaking and allowing to stand for 5 minutes it is diluted with water to 500 c.c. and mixed. This solution is now compared in a Duboscq colorimeter with a ½ normal potassium-bichromate solution. The latter solution has in a layer 8 mm. thick exactly the same intensity of color as a layer 8.1 mm. thick of a solution of 10 milligrams creatinine after the addition of 15 c.c. picric-acid solution and 5 c.c. NaOH solution and dilution to 500 c.c. The calculations are simple. For example, in case the urine tested in a layer 7.2 mm. thick has the same color as the dichromate solution in a layer 8 mm. thick, then the quantity of creatinine in 10 c.c. of the urine will be $=\frac{8.1}{7.2}\times 10$, or 11.25 milligrams. This method is not only simple, but also, according to Folin, Hoogenhuyze and Verploegh, gives much more trustworthy results than Neubauer's method.

In regard to other methods, see the works of Kolisch and Gregor.[2]

Xanthocreatinine, $C_5H_{10}N_4O$. This body, which was first prepared from meat extract by Gautier, has been found by Monari in dog's urine after the injection of creatinine into the abdominal cavity, and in human urine after several hours of exhausting marching. According to Colasanti it occurs to a relatively greater extent in lion's urine. Stadthagen[3] considers the xanthocreatinine isolated from human urine after strenuous muscular activity as impure creatinine.

Xanthocreatinine forms thin sulphur-yellow plates, similar to cholesterin, which have a bitter taste. It dissolves in cold water and in alcohol, and gives a crystalline compound with hydrochloric acid and a double compound with gold and platinum chloride. It gives a compound with zinc chloride, which crystallizes in fine needles. Xanthocreatinine has a poisonous action.

Uric Acid, $\bar{U}r$, $C_5H_4N_4O_3$, 2, 6, 8-trioxypurine =



has

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[1] Zeitschr. f. physiol. Chem., 41.

[2] Kolisch, Centralbl. f. innere Med., 1895; Gregor, Zeitschr. f. physiol. Chem., 31.

[3] Gautier, Bull. de l'acad. de méd. (2) 15, and Bull. de la soc. chim. (2), 48: Monari, Maly's Jahresber., 17; Colasanti, Arch. ital. d. Biologie, 15, Fasc. 3; Stadthagen, Zeitschr. f. klin. Med., 15.

been prepared synthetically by **Horbaczewski** by fusing urea and glycocoll or by heating trichlorlactic-acid amide with an excess of urea. **Behrend** and **Roosen** prepared it from isodialuric acid and urea; it is also readily produced from isouric acid on boiling with hydrochloric acid (E. **Fischer** and **Tüllner**), and finally E. **Fischer** and **Ach**[1] have prepared uric acid from pseudouric acid by heating with oxalic acid to 145° C.

On strongly heating uric acid it decomposes with the formation of *urea*, *hydrocyanic acid*, *cyanuric acid*, and *ammonia*. On heating with concentrated hydrochloric acid in sealed tubes to 170° C. it splits into *glycocoll*, *carbon dioxide*, and *ammonia*. By the action of oxidizing agents splitting and oxidation take place, and either monoureides or diureides are produced. By oxidation with lead peroxide, *carbon dioxide*, *oxalic acid*, *urea*, and *allantoin*, which last is glyoxyldiureide, are produced (see below). By oxidation with nitric acid in the cold, *urea* and a monoureide, the mesoxalyl urea, or *alloxan*, are obtained, $C_5H_4N_4O_3+O+H_2O=C_4H_2N_2O_4+(NH_2)_2CO$. On warming with nitric acid, alloxan yields carbon dioxide and oxalyl urea, or *parabanic acid*, $C_3H_2N_2O_3$. By the addition of water the parabanic acid passes into *oxaluric acid*, $C_3H_4N_2O_4$, traces of which are found in the urine and which easily splits into oxalic acid and urea. In alkaline solution uric acid may, by taking up water and oxygen, be transformed into a new acid, uroxanic acid, $C_5H_8N_4O_6$, which may then be changed into oxonic acid, $C_4H_5N_3O_4$.[2] Uric acid may, as F. and L. **Sestini** as well as **Gerard** have shown, undergo bacterial fermentation with the formation of urea. According to **Ulpiani** and **Cingolani**,[3] uric acid is quantitatively split hereby into urea and carbon dioxide, according to the equation

$$C_5H_4N_4O_3+2H_2O+3O=3CO_2+2CO(NH_2)_2.$$

Uric acid occurs most abundantly in the urine of birds and of scaly amphibians, in which animals the greater part of the nitrogen of the urine appears in this form. Uric acid occurs frequently in the urine of carnivorous mammalia, but is sometimes absent; in urine of herbivora it is habitually present, though only as traces; in human urine it occurs in greater but still small and variable amounts. Traces of uric acid are also found in several organs and tissues, as in the spleen, lungs, heart, pancreas, liver (especially in birds), and in the brain. It habitually occurs in the blood of birds. Traces have been found in human blood under normal conditions. Under pathological conditions it occurs to an increased extent in the blood, as in pneumonia and nephritis, but especially in leucæmia and sometimes also in arthritis. Uric acid also occurs in large quantities in "chalk-stones," certain urinary calculi, and in guano. It has also been detected in the urine of insects and certain snails, as also in the wings (which it colors white) of certain butterflies (HOPKINS).[1]

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[1] Horbaczewski, Monatshefte f. Chem., **6** and **8**; Behrend and Roosen, Ber. d. d. chem. Gesellsch., **21**; Fischer and Tüllner, *ibid.*, **35**; Fischer and Ach, *ibid.*, **28**.

[2] See Sundwik, Zeitschr. f. physiol. Chem., **20** and **41**; also Behrend, Annal. d. Chem. u. Pharm., **333**.

[3] See Chem. Centralbl., 1903, where the other investigators are cited, and Centralbl. f. Physiol., **19**.

The amount of uric acid eliminated with human urine is subject to considerable individual variation, but amounts on an average to 0.7 gram per day on a mixed diet. The ratio of uric acid to urea varies considerably with a mixed diet, but is on an average 1:50–1:70. In new-born infants and in the first days of life the elimination of uric acid is relatively increased, and the relation between uric acid and urea has been found to be 1:6.42–17.1.

We used to ascribe an increasing action upon the elimination of uric acid to protein food, but the investigations of HIRSCHFELD, ROSENFELD and ORGLER, SIVÉN, BURIAN and SCHUR,[2] and many others have positively proved that a diet rich in protein does not itself increase the elimination of uric acid, but only according to the amount of nucleins or purine bodies contained therein. The common statement that the elimination of uric acid is smaller with a vegetable diet than with an animal diet, when the quantity may be 2 grams or more per twenty-four hours, is explained by this.[3]

The statements in regard to the influence of other circumstances, as also of different substances, on the elimination of uric acid are rather contradictory. This is in part due to the fact that the older investigators used an inaccurate method (HEINTZ), and also that the extent of uric-acid elimination is dependent in the first place upon the individuality. Thus the statements in regard to the action of drinking-water[4] and of alkalies[5] are very contradictory. Certain medicines, such as quinine and atropine, diminish, while others, such as pilocarpine and also, as it seems, salicylic acid,[6] increase the elimination of uric acid.

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[1] Philos. Trans. Roy. Soc., 186, B, 661.

[2] See the extensive review of the literature in Wiener, "Die Harnsäure," in Ergebnisse der Physiologie, 1, Abt. 1, 1902.

[3] J. Ranke, Beobachtungen und Versuche über die Ausscheidung der Harnsäure, etc. (München, 1858); Mares, Centralbl. f. d. med. Wissensch., 1888; Horbaczewski, Wien. Sitzungsber., 100, Abt. 3, 1891. In regard to the action of various diets the reader is referred to the above-cited authors, and especially to A. Hermann, Arch. f. klin. Med., 43, and Camerer, Zeitschr. f. Biologie, 33, and Folin, Amer. Journ. of Physiol., 13.

[4] See Schöndorff, Pflüger's Arch., 46, which contains the pertinent literature.

[5] See Clar, Centralbl. f. d. med. Wissensch., 1888; Haig, Journ. of Physiol., 8; and A. Hermann, Arch. f. klin. Med., 43.

[6] See Bohland, cited from Maly's Jahresber., 26; Schreiber and Zaudy, *ibid.*, 30.

Little is known with positiveness in regard to the elimination of uric acid in disease. In acute diseases with crises the elimination of uric acid is increased after the crisis, while the older statements that the uric acid is habitually increased in fevers has been contradicted by many. The statements in regard to the elimination of uric acid in gout and nephritis are also uncertain and contradictory. In leucæmia the elimination is increased absolutely as well as relatively to the urea, and the relationship between the uric acid and urea (total nitrogen recalculated as urea) may be even 1:9, while under normal conditions, according to different investigators, it is 1:40 to 66 to 100.[1]

Formation of Uric Acid in the Organism. Since HORBACZEWSKI first showed that uric acid could be produced by oxidation from the nuclein-rich spleen-pulp or nucleins outside of the body, he also showed that nucleins when introduced into the animal body caused an increase in the elimination of uric acid. These observations have been confirmed, and at the same time developed by the work of a great number of investigators, and we are sure that uric acid can be produced from purine bodies either outside or inside the animal body, and also that food rich in nucleins (especially the thymus gland) increases the elimination of uric acid and purine bases (alloxuric bases [2]). The original view of HORBACZEWSKI, that the nucleins do not directly cause an increased elimination of uric acid, but indirectly by causing a leucocytosis with a consequent destruction of leucocytes, has been nearly generally discarded. At present it is considered that a direct formation of uric acid from the nucleins takes place by the transformation of the purine bases of the nucleins into uric acid.

The uric acid, in so far as it is produced from nuclein bases, is in part derived from the nucleins of the destroyed cells of the body and in part from the nucleins or free purine bases introduced with the food. It is therefore possible to admit with BURIAN and SCHUR [3] of a double origin for the uric acid as well as the urinary purines (all purine bodies of the urine, including the uric acid), namely, an *endogenous* and an *exogenous* origin. BURIAN and SCHUR attempted to determine the quantity of endogenous urinary purines by feeding with sufficient food, but as free as possible from purine bodies, and they found that this quantity was constant for every individual, while it was variable for different persons. The observations of SIVÉN, ROCKWOOD,[4] and others have also led to the same results. Other investigators, such as **Schreiber** and **Waldvogel**, **Loewi**, and **Folin**,[1] have arrived at somewhat different results, or they draw differentde ductions from their observations; still this does not change the essential fact, that the uric acid originating from the nucleins is partly endogenous and partly exogenous, and that the amount of endogenous uric acid is only very slightly dependent upon the protein content of the food.

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[1] In regard to the extensive literature on the elimination of uric acid in disease we must refer to special works on internal diseases.

[2] As it is not within the scope of this book to enter into a discussion of the numerous researches on this subject, we will refer to Wiener, "Die Harnsäure," Ergebnisse der Physiol., **1**, Abt. 1, 1902.

[3] Pflüger's Arch., **80**, **87**, and **94**.

[4] Amer. Journ. of Physiol., **12**.

In man and other mammalia the greatest amount if not all of the uric acid originates from the nucleins or their purine bases. This formation of uric acid seems to be of an enzymotic kind. After it was shown that certain organs, such as the liver and spleen, had the power of converting oxypurines into uric acid in the presence of oxygen (**Horbaczewski**, **Spitzer** and **Wiener**[2]), recently **Schittenhelm**, **Burian**, **Jones** and **Partridge**,[3] by more careful investigations have shown that enzymes of different kinds act together. By means of the two deamidizing enzymes *adenase* and *guanase* the adenine and guanine are transformed into hypoxanthine and xanthine respectively, and from the latter by means of an oxidizing enzyme, called *xanthine oxidase* by **Burian**, the uric acid is formed. The deamidizing enzymes seem to be present in most organs, yet there exists, in this regard, a marked difference between certain animals; thus guanase occurs in the ox-spleen but not in the pig-spleen (**Jones** and **Winterintz**). The oxidase occurs especially in the spleen (though not in the dog spleen, according to **Schittenhelm**) and liver, but also in the muscles and lungs. Still, as **Schittenhelm**[4] has especially shown, a very marked difference exists in animals, and the activity of the organs of various animals requires a very thorough investigation.

Jones and **Austrian**[5] have carried on investigations on the occurrence in different organs of pigs, dogs, and rabbits, of enzymes taking part in the nuclein metabolism. The occurrence of these enzymes in the liver is of special interest. In the above-mentioned animals and in the ox they found the following: The beef-liver contains guanase, adenase, and xanthine oxidase, and produces uric acid from guanine as well as from adenine. Guanase is absent from the pig-liver, while adenase and xanthine oxidase are present. In these animals the liver forms uric acid from adenine but not from guanine. The rabbit-liver does not contain any adenase and hence uric acid is formed only from guanase. while the dog-liver, on the contrary, which contains guanase but neither adenase nor xanthine oxidase, cannot form uric acid from guanine nor from adenine.

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[1] Schreiber and Waldvogel, Arch. f. exp. Path. u. Pharm., **42**; O. Loewi, *ibid.*, **44** and **45**; Folin, Amer. Journ. of Physiol., **13**.

[2] See foot-note 2, page 570.

[3] Schittenhelm, Zeitschr. f. physiol. Chem., **42**, **43**, **45**, and **46**; Burian, *ibid.*, **43**; Jones and Partridge, *ibid.*, **42**; Jones and Winternitz *ibid.*, **44**; Jones, *ibid.*, **45**.

[4] Zeitschr. f. physiol. Chem., **46**.

[5] Zeitschr. f. physiol. Chem., **48**.

In birds the conditions are different. v. MACH[1] has shown that in these animals a part of the uric acid may be formed from the purine bodies. The chief quantity of uric acid, however, is undoubtedly formed in birds by synthesis.

The formation of uric acid in birds is increased by the administration of ammonium salts (v. SCHRÖDER), and urea acts in a similar manner in these animals (MEYER and JAFFÉ). MINKOWSKI observed in geese with extirpated livers a very significant decrease in the elimination of uric acid, while the elimination of ammonia was increased to a corresponding degree. This indicates a participation of ammonia in the formation of uric acid in the organism of birds; and as MINKOWSKI has also found after the extirpation of the liver that considerable amounts of lactic acid occur in the urine, it is probable that the uric acid in birds is produced in the liver by synthesis perhaps from lactic acid and ammonia; although, as SALASKIN and ZALESKI and LANG have shown, after the extirpation of the liver primarily an increase in the formation of lactic acid occurs and this causes an increase in the elimination of ammonia (neutralization ammonia). The direct proof for the uric-acid formation from ammonia and lactic acid in the liver of birds has been given by KOWALEWSKI and SALASKIN[2] by means of blood-transfusion experiments on geese with extirpated livers. They observed a relatively abundant formation of uric acid after the addition of ammonium lactate and a still greater formation after arginine. They not only consider ammonium lactate but also amino-acids as substances from which the uric acid can be produced in the liver by synthesis. Of these leucine, glycocoll, and aspartic acid increase the elimination of uric acid in birds (v. KNIERIEM[3]), but whether they are first decomposed with the splitting off of ammonia is still unknown.

The possibility of a formation of uric acid from lactic acid has been shown in another manner by WIENER,[4] namely, by feeding birds with urea and lactic acid and different non-nitrogenous substances, oxy-, keto-, and dibasic acids of the aliphatic series. The dibasic acids, with a chain of 3 carbon atoms or their ureides, showed themselves most active as uric-acid formers, and WIENER is therefore of the opinion that the active substances must first be converted into dibasic acids. By the attachment of a urea residue the corresponding ureide is produced, according to WIENER, and from this the uric acid is derived by the attachment of a second urea residue.

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[1] Arch. f. exp Path. u. Pharm., **24**.

[2] v. Schröder, Zeitschr. f. physiol. Chem., **2**; Meyer and Jaffé, Ber. d. d. Chem. Gesellsch., **10**; Minkowski, Arch. f. exp. Path. u. Pharm., **21** and **31**; Salaskin and Zaleski, Zeitschr. f. physiol. Chem., **29**; Lang, *ibid.*, **32**; Kowalewski and Salaskin, *ibid.*, **33**.

[3] Zeitschr. f. Biologie, **13**.

[4] Hofmeister's Beiträge, **2**. See also Arch. f. exp. Path. u. Pharm., **42**, and Ergebnisse d. Physiol., **1**, Abt. 1, 1902.

Among the substances tested, only tartronic acid and its ureide, dialuric acid, have shown themselves active in the experiments with the isolated organs, and WIENER therefore also considers that the other acids must be first converted into tartronic acid by oxidation or reduction. From lactic acid, $CH_3.CH(OH).COOH$, we first obtain tartronic acid, $COOH.CH(OH).COOH$, which by the attachment of a urea residue forms dialuric acid, $CO<\begin{matrix}NH-CO\\NH-CO\end{matrix}>CHOH$, and from this, by the attachment of a second urea residue, uric acid is formed.

We cannot give any positive answer as to the question whether uric acid is formed by synthesis also in man and other mammalia. WIENER has in part reported experiments which seem to indicate a synthetic uric-acid formation in the isolated mammalian liver, and he has also obtained an increase in the uric-acid elimination, although only a slight one, after feeding lactic acid and dialuric acid to man. According to BURIAN [1] we have for the present no proof of a synthetical formation of uric acid in the mammalian liver. Dialuric acid and tartronic acid, according to him, do not cause any marked uric-acid formation with extracts of the ox-liver in the absence of purine bases; on the contrary they accelerate the enzymotic oxidation of purine bases and hence, according to BURIAN, this explains, perhaps, the increase in uric-acid elimination.

The liver seems to be the organ in birds where the synthetical formation of uric acid occurs, and the fact that it was possible for MINKOWSKI [2] to arrest the uric-acid formation by the extirpation of the liver apparently shows that the liver is the only organ taking part in this synthesis. If a synthesis of uric acid also occurs in man and other mammalia we must consider the liver as at least one of the organs taking part in the work, as shown by WIENER'S investigations. The liver, spleen, and muscles are considered as the most important organs for the oxidative uric-acid formation from nucleins and purine bases, but it must not be forgotten that these organs in various animals have a somewhat different behavior in this regard.

Uric acid when introduced into the mammalian organism is, as first shown by WÖHLER and FRERICHS for the dog and later substantiated by several experimenters,[3] in great part destroyed and more or less completely changed into urea. This does not seem to be the same for all animals. In rabbits, according to WIENER, the uric acid is destroyed with the formation of glycocoll as an intermediate step. The statements are very contradictory in regard to carnivora. According to an older view, which has received support by the recent investigations of SALKOWSKI, a part of the uric acid introduced into dogs is eliminated as allantoin, which is also true according to MENDEL and BROWN for cats. The correctness of this view is denied by WIENER, POHL and PODUSCHKA, but the recent investigations of MENDEL and WHITE [1] give further proofs of its correctness. The possibility of a uric-acid destruction in man, with allantoin as an intermediary step, cannot, for the same reasons, be denied.

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[1] Zeitschr. f. physiol. Chem., **43**.

[2] l. c.

[3] Wöhler and Frerichs, Annal. d. Chem. u. Pharm., **65**. See also Wiener, Ergebnisse der Physiologie, **1**, Abt. 1.

The demolition of uric acid seems to be possible, according to the numerous researches of CHASSEVANT and RICHET, ASCOLI, JACOBY, WIENER, SCHITTENHELM, BURIAN, ALMAGIA and PFEIFFER,[2] in several organs, such as the liver, kidneys, muscle, and bone-marrow, although its behavior differs in various animals.

From this power of the various organs of destroying uric acid it follows that the quantity of uric acid eliminated is not a sure indication of the amount of the acid formed. We must admit, therefore, that a part of the uric acid formed in the body is destroyed in a similar manner to that introduced from without. BURIAN and SCHUR [3] have indeed suggested a factor, the so-called "integral factor," with which the quantity of uric acid eliminated in the twenty-four hours must be multiplied in order to find the quantity of uric acid formed during this time. According to them, carnivora eliminate unchanged about $\frac{1}{20}-\frac{1}{30}$ of the uric acid introduced into the circulation, rabbits about $\frac{1}{5}$, and man $\frac{1}{2}$.

Properties and Reactions of Uric Acid. Pure uric acid is a white, odorless, and tasteless powder consisting of very small rhombic prisms or plates. Impure uric acid is easily obtained as somewhat larger, colored crystals.

In rapid crystallization, small, thin, four-sided, apparently colorless, rhombic prisms are formed, which can be seen only by the aid of the microscope, and these sometimes appear as spools because of the rounding of their obtuse angles. The plates are sometimes six-sided, irregularly developed; in other cases they are rectangular with partly straight and partly jagged sides; and in other cases they show still more irregular forms, the so-called dumb-bells, etc. In slow crystallization, as when the urine deposits a sediment or when treated with acid, large, invariably colored crystals separate. Examined with the microscope these crystals always appear yellow or yellowish brown in color. The most common type is the whetstone shape, formed by the rounding off of the obtuse angles of the rhombic plate. The whetstones are generally connected together, two or more crossing each other. Besides these forms, rosettes of prismatic crystals, irregular crosses, brown-colored rough masses of broken-up crystals and prisms occur, as well as other forms.

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[1] Wiener, Arch. f. exp. Path. u. Pharm., **40** and **42**, and Ergebnisse der Physiologie, **1**, Abt. 1; Pohl, Arch. f. exp. Path. u. Pharm., **48**; Poduschka, *ibid.*, **44**; Salkowski, Zeitschr. f. physiol. Chem., **35**, and Ber. d. d. Chem. Gesellsch., **9**; Mendel and Brown, Amer. Journ. of Physiol., **3**; Mendel and White, *ibid.*, **12**.

[2] Chassevant et Richet, Compt. rend. Soc. biolog., **49**; Ascoli, Pflüger's Arch., **72**; Jacoby, Virchow's Arch., **157**; Wiener, Arch. f. exp. Path. u. Pharm., **42**, and Centralbl. f. Physiol., **18**; Schittenhelm, Zeitschr. f. physiol. Chem., **43** and **45**; Burian, *ibid.*, fmeister's Beiträge, **7**; Pfeiffer, *ibid.*, **7**.

Uric acid is insoluble in alcohol and ether; it is rather easily soluble in boiling glycerine, but very insoluble in cold water, in 39 480 parts at 18° C. (His and Paul). At this temperature, according to them, 9.5 per cent of the uric acid is dissociated in the saturated solution. Because of the reduction in the dissociation on the addition of strong acids uric acid is soluble with difficulty in the presence of mineral acids. It is soluble in a warm solution of sodium diphosphate, and in the presence of an excess of uric acid, monophosphate and acid urate are produced. It is ordinarily assumed that sodium diphosphate forms a solvent for the uric acid in the urine, but according to Smale the monophosphate has only a slight solvent action. According to Rüdel [1] urea is an important solvent, but this statement has not been confirmed by the observations of His and Paul. Uric acid is not only dissolved by alkalies and alkali carbonates, but also by several organic bases, such as ethylamine and propylamine, piperidine and piperazine. Uric acid dissolves without decomposing in concentrated sulphuric acid. It is completely precipitated from the urine by picric acid (Jaffé [2]). Uric acid gives a chocolate-brown precipitate with phosphotungstic acid in the presence of hydrochloric acid.

Uric acid is dibasic and correspondingly forms two series of salts, neutral and acid. Of the alkali urates the neutral potassium and lithium salts are most easily soluble and the ammonium salt dissolves with difficulty. The acid alkali urates are very insoluble and separate as a sediment (*sedimentum lateritium*) from concentrated urine on cooling. The salts with alkaline earths are very insoluble.

If a little uric acid in substance is treated on a porcelain dish with a few drops of nitric acid. the uric acid dissolves on warming with a strong development of gas, and after thoroughly drying on the water-bath a beautiful red residue is obtained, which turns a purple-red (ammonium purpurate or murexide) on the addition of a little ammonia. If, instead of the ammonia, we add a little caustic soda (after cooling), the color becomes deeper blue or bluish violet. This color disappears quickly on warming, differing from certain xanthine bodies. This reaction is called the *murexide test*.

If uric acid is converted into alloxan by the careful action of nitric acid and the excess of acid carefully expelled, on treating this with a few drops of concentrated sulphuric acid and commercial benzene (containing thiophene) a beautiful blue coloration is obtained (DENIGÈS' reaction [1]).

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[1] His, Jr., and Paul, Zeitschr. f. physiol. Chem., **31**; Smale, Centralbl. f. Physiol., **9**; Rüdel, Arch. f. exp. Path. u. Pharm., **30**.

[2] Zeitschr. f. physiol. Chem., **10**.

Uric acid does not reduce an alkaline solution of bismuth, while, on the contrary, it reduces an alkaline cupric-hydrate solution. In the presence of only a little copper salt we obtain a white precipitate consisting of cuprous urate. In the presence of more copper salt red cuprous oxide separates. The compound of uric acid with cuprous oxide is formed when copper salts are reduced by dextrose or a bisulphite in alkaline solution in the presence of a sufficient amount of urate.

If a solution of uric acid in water containing alkali carbonate is treated with magnesium mixture and then a silver-nitrate solution added, a gelatinous precipitate of silver-magnesium urate is formed. If a drop of uric acid dissolved in sodium carbonate is placed on a piece of filter-paper which has been previously treated with silver-nitrate solution, a reduction of silver oxide occurs, producing a brownish-black or, in the presence of only 0.002 milligram of uric acid, a yellow spot (SCHIFF's test).

The precipitation of free uric acid from its alkali salts by means of acids can be prevented to some extent by the presence of thymic acid or nucleic acid (GOTO [2]). It is questionable whether this is of any physiological importance.

Preparation of Uric Acid from Urine. Filtered normal urine is treated with 20–30 c.c. of 25 per cent hydrochloric acid for each liter of urine. After forty-eight hours collect the crystals and purify them by redissolving in dilute alkali, decolorizing with animal charcoal and reprecipitating with hydrochloric acid. Large quantities of uric acid are easily obtained from the excrements of serpents by boiling them with dilute caustic potash (5 per cent) until no more ammonia is developed. A current of carbon dioxide is passed through the filtrate until it barely has an alkaline reaction; dissolve the separated and washed acid potassium urate in caustic potash, and precipitate the uric acid in the filtrate by addition of an excess of hydrochloric acid.

Quantitative Estimation of Uric Acid in the Urine. As the older method suggested by HEINTZ, even after recent modifications, gives inaccurate results, it will not be considered here.

SALKOWSKI and LUDWIG's [3] method consists in precipitating by silver nitrate the uric acid from the urine previously treated with magnesium mixture, and weighing the uric acid obtained from the silver precipitate. Uric acid determinations by this method are often performed according to the suggestion of E. LUDWIG, which requires the following solutions:

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[1] Journ. de Pharm. et de Chim., **18**. Cited from Maly's Jahresber., **18**.

[2] Zeitschr. f. physiol. Chem., **30**.

[3] Salkowski, Virchow's Arch., **52**; Pflüger's Arch., **5**; Salkowski, Laboratory Manual of Physiol. and Path. Chem., translated by Orndorff, 1904; Ludwig, Wien. med. Jahrbuch, 1884, and Zeitschr. f. anal. Chem., **24**.

1. An AMMONIACAL SILVER-NITRATE SOLUTION, which contains in 1 liter 26 grams of silver nitrate and a quantity of ammonia sufficient to redissolve completely the precipitate produced by the first addition of ammonia. 2. MAGNESIA MIXTURE. Dissolve 100 grams of crystallized magnesium chloride in water, add ammonia until the liquid smells strongly of it, and enough ammonium chloride to dissolve the precipitate; then dilute the solution to 1 liter. 3. SODIUM-SULPHIDE SOLUTION. Dissolv e10 grams of caustic soda which is free from nitric acid and nitrous acid in 1 liter of water. One half of this solution is completely saturated with sulphuretted hydrogen and then mixed with the other half.

The concentration of the three solutions is so arranged that 10 c.c. of each is sufficient for 100 c.c. of the urine.

100–200 c.c., according to concentration, of the filtered urine freed from protein (by boiling after the addition of a few drops of acetic acid) is poured into a beaker. In another vessel mix 10–20 c.c. of the silver solution with 10–20 c.c. of the magnesia mixture and add ammonia, and when necessary also some ammonium chloride, until the mixture is clear. This solution is added to the urine while stirring, and the mixture allowed to stand quietly for half an hour. The precipitate is collected on a filter, washed with ammoniacal water, and then returned to the same beaker by the aid of a glass rod and a wash-bottle, without destroying the filter. Now heat to boiling 10–20 c.c. of the alkali-sulphide solution, which has previously been diluted with an equal volume of water, and allow this solution to flow through the above filter into the beaker containing the silver precipitate; wash with boiling water, and warm the contents of the beaker on a water-bath for a time, stirring constantly. After cooling, filter into a porcelain dish, wash the filter with boiling water, acidify the filtrate with hydrochloric acid, evaporate it to about 15 c.c., add a few drops more of hydrochloric acid, and allow it to stand for twenty-four hours. The uric acid which has crystallized is collected on a small weighed filter, washed with water, alcohol, ether, and carbon disulphide, dried at 100–110° C., and weighed. For each 10 c.c. of aqueous filtrate we must add 0.00048 gram uric acid to the quantity found directly. Instead of the weighed filter-paper a glass tube filled with glass wool as described in other handbooks may be substituted (LUDWIG). Too intense or too long continued heating with the alkali sulphide must be prevented, otherwise a part of the uric acid may be decomposed.

SALKOWSKI deviates from this procedure by precipitating the urine first with a magnesium mixture (50 c.c. to 200 c.c. urine), filling up to 300 c.c., and filtering. Of the filtrate, 200 c.c. is precipitated by 10–15 c.c. of a 3 per cent silver-nitrate solution. The silver precipitate is shaken with 200–300 c. c. of water acidified with a few drops of hydrochloric acid, decomposed by sulphuretted hydrogen, heated to boiling, the silver-sulphide precipitate boiled with fresh water, filtered, the filtrate concentrated to a few cubic centimetres, treated with 5–8 drops of hydrochloric acid, and allowed to stand until the next day.

HOPKINS' method is based on the fact that the uric acid is completely precipitated from the urine as ammonium urate on saturating with ammonium chloride. The uric acid can either be weighed after being set free by hydrochloric acid or it can be determined in several ways—by titration with potassium permanganate or by the KJELDAHL method. Several modifications of this method have been worked out by FOLIN, FOLIN and SCHAF-

FER, WÖRNER, and JOLLES.[1] The last named converts the uric acid into urea by oxidation with potassium permanganate in sulphuric-acid solution and then determines the quantity of this by sodium hypobromite. Of these methods we shall describe only that suggested by FOLIN-SCHAFFER.

Folin-Schaffer Method. Treat 300 c.c. urine with 75 c.c. of a solution containing 500 grams of ammonium sulphate, 5 grams of uranium acetate, and 60 c.c. of 10 per cent acetic acid in a liter, and filter after five minutes. This removes an unknown constituent of the urine (a protein substance) which would otherwise contaminate the uric acid. Take 125 c.c. of the filtrate (corresponding to 100 c.c. of the urine) and add 5 c.c. of concentrated ammonia. After twenty-four hours the precipitate is filtered off and washed free from chlorine on the filter by means of an ammonium-sulphate solution. The precipitate is washed off the filter by water (total 100 c.c.) into a flask, treated with 15 c.c. of concentrated sulphuric acid, and titrated at 60–63° C. with N/20 potassium-permanganate solution. Each cubic centimeter of this solution corresponds to 3.75 milligrams uric acid. Because of the solubility of the ammonium urate a correction of 3 milligrams must be added for every 100 c.c. of the urine.

In regard to the numerous other methods for estimating uric acid, we must refer to special works on the subject, and especially to HUPPERT-NEUBAUER.

Purine Bases (ALLOXURIC BASES). The alloxuric bases (purine bases) found in human urine are *xanthine, guanine, hypoxanthine, adenine, paraxanthine, heteroxanthine, episarkine, epiguanine, 1-methylxanthine,* and *carnine.* The occurrence of guanine and carnine (POUCHET) is, according to KRÜGER and SALOMON,[2] not positively shown. The quantity of these bodies in the urine is extremely small and varies in different individuals. FLATOW and REITZENSTEIN [3] found 15.6–45.1 milligrams in the urine voided during twenty-four hours. The quantity of alloxuric bases in the urine is increased regularly after feeding with nucleus nucleins or food rich in nucleins, and after free destruction of leucocytes. The quantity is especially increased in leucæmia. We have a number of observations on the elimination of these bodies in different diseases, but they are hardly trustworthy on account of the inaccuracy of the methods used in the determinations. It must also be remarked that the three alloxuric bases, heteroxanthine, paraxanthine, and 1-methylxanthine, which form the chief mass of the alloxuric bases of the urine, are derived, according to the investigations of ALBANESE, BONDZYNSKI and GOTTLIEB, E. FISCHER, M. KRÜGER and G. SALOMON, and

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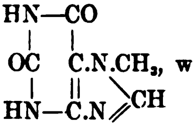
[1] Hopkins, Journ. of Path. and Bact., 1893, and Proceed. Roy. Soc., **52**; Folin, Zeitschr. f. physiol. Chem., **24**; Folin and Schaffer, *ibid.*, **32**; Wörner, *ibid.*, **29**; Jolles, *ibid.*, **29**, and Wien. med. Wochenschr., 1903.

[2] Zeitschr. f. physiol. Chem., **24**; Pouchet, "Contributions à la connaissance des matières extractives de l'urine." Thèse Paris, 1880. Cited from Huppert-Neubauer, 333 and 335.

[3] Deutsch. med. Wochenschr., 1897.

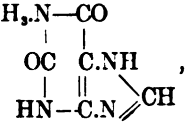
SCHMIDT[1] from the theobromine, caffeine, and theophylline which occur in the food. With the purine bases we must also differentiate between those of endogenous and those of exogenous origin.[2] As the four true nuclein bases and carnine have been treated in Chapters V and XI, it only remains to describe the special urinary purine bodies.



$$\begin{array}{lll} HN & - & CO \\ | & & | \\ OC & & C.N.CH_3 \\ | & & \| \quad \searrow CH \\ HN & - & C.N \nearrow \end{array}$$

Heteroxanthine, $C_6H_6N_4O_2$ = 7-monomethylxanthine, was first detected in the urine by SALOMON. It is identical with the monomethylxanthine which passes into the urine after feeding with theobromine or caffeine. SALOMON and NEUBERG[3] found heteroxanthine in the urine of a dog fed entirely upon meat, and this was probably formed by a methylation in the body.

Heteroxanthine crystallizes in shining needles and dissolves with difficulty in cold water (1592 parts at 18° C.). It is readily soluble in ammonia and alkalies. The crystalline sodium salt is insoluble in strong caustic alkali (33 per cent) and dissolves with difficulty in water. The chloride crystallizes beautifully, is relatively insoluble, and is readily decomposed into the free base and hydrochloric acid by water. Heteroxanthine is precipitated by copper sulphate and bisulphite, mercuric chloride, basic lead acetate and ammonia, and by silver nitrate. The silver compound dissolves rather easily in dilute, warm nitric acid; it crystallizes in small rhombic plates or prisms, often grown together, forming characteristic crosses. Heteroxanthine does not give the xanthine reaction, but does give WEIDEL's reaction, according to FISCHER (see Chapter V).



$$\begin{array}{lll} CH_3.N & - & CO \\ | & & | \\ OC & & C.NH \\ | & & \| \quad \searrow CH \\ HN & - & C.N \nearrow \end{array}$$

1-Methylxanthine, $C_6H_6N_4O_2$, was first isolated from the urine and studied by KRÜGER, and then by KRÜGER and SALOMON.[4] It is difficultly soluble in cold water, but readily soluble in ammonia and caustic soda, and does not give an insoluble sodium compound. It is readily soluble in dilute acids, and it crystallizes from its acetic-acid solution in thin, generally hexagonal plates. The chloride is decomposed into the base and hydrochloric acid by water. 1-methylxanthine gives crystalline double salts with platinum and gold. It is not precipitated by basic lead acetate, nor when pure by basic lead acetate and ammonia. With ammonia and silver nitrate it gives a gelatinous precipitate. The silvr-nitrate compound crystallized from nitric acid forms rosettes of united needles. With the xanthine test with nitric acid it gives an orange coloration on the addition of caustic soda. It gives WEIDEL's reaction (according to FISCHER) beautifully.

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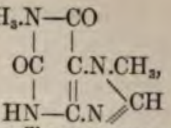
[1] Albanese, Ber. d. d. chem. Gesellsch., **32**; Arch. f. exp. Path. u. Pharm., **35**; Bondzynski and Gottlieb, *ibid.*, **36**, and Ber. d. deutsch. chem. Gesellsch., **28**; E. Fischer, *ibid.*, **30**, 2405; Krüger and Salomon, Zeitschr. f. physiol. Chem., **26**; Krüger and Schmidt, Ber. d. d. chem. Gesellsch., **32**, and Arch. f. exp. Path. u. Pharm., **45**.

[2] See Burian and Schur, foot-note 3, page 570, and Kaufmann and Mohr, Deutsch. Arch. f. klin. Med., **74**.

[3] Salkowski's Festschrift, 1904.

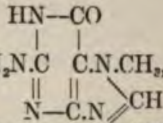
[4] Krüger, Arch. f. (Anat. u.) Physiol., 1894; Krüger and Salomon, Zeitschr. f physiol. Chem., **24**.

Paraxanthine, $C_7H_8N_4O_2$ = 1.7-dimethylxanthine, 

$$\begin{array}{lll} CH_3.N & — & CO \\ \mid & & \mid \\ OC & & C.N.CH_3 \\ \mid & & \| \quad \searrow \\ HN & — & C.N \nearrow CH \end{array}$$

urotheobromine (Thudichum), was first isolated from the urine by Thudichum and Salomon.[1] It crystallizes beautifully in six-sided plates or in needles. The sodium compound crystallizes, in rectangular plates or prisms and, like the heteroxanthine-sodium compound, is insoluble in 33 per cent caustic-soda solution. The sodium compound separates in a crystalline state on neutralizing its solution in water. The chloride is readily soluble and is not decomposed by water. The chloroplatinate crystallizes very beautifully. Mercuric chloride precipitates it only when added in excess and after a long time. The silver-nitrate compound separates as white silky crystals from hot nitric acid on cooling. It gives Weidel's reaction, but not the xanthine test, with nitric acid and alkali.

Episarkine is the name given by Balke to a purine body occurring in human urine. The same body has been observed by Salomon[2] in pigs' and dogs' urine, as well as in urine in leucæmia. Balke gives $C_4H_6N_3O$ as the probable formula for episarkine. It is nearly insoluble in cold water, dissolves with difficulty in hot water, but may be obtained therefrom as long fine needles. Episarkine does not give the xanthine reaction with nitric acid nor Weidel's reaction. With hydrochloric acid and potassium chlorate it gives a white residue which turns violet with ammonia. It does not form any insoluble sodium compound. The silver compound is difficultly soluble in nitric acid. Episarkine is possibly identical with epiguanine.

Epiguanine, $C_6H_7N_5O$ = 7-methylguanine, 

$$\begin{array}{lll} HN & — & CO \\ \mid & & \mid \\ H_2N.C & & C.N.CH_3 \\ \| & & \| \quad \searrow \\ N & — & C.N \nearrow CH \end{array}$$

was first prepared from the urine by Krüger.[3] It is crystalline and difficultly soluble in hot water or ammonia. It crystallizes from a hot 33 per cent caustic-soda solution on cooling in broad shining crystals and dissolves readily in hydrochloric or sulphuric acid. It gives a characteristic chloroplatinate crystallizing in six-sided prisms. It is precipitated neither by basic lead acetate nor by basic lead acetate and ammonia. Silver nitrate and ammonia give a gelatinous precipitate. It responds to the xanthine test with nitric acid and alkali. According to Fischer it acts like episarkine with Weidel's test.

In preparing alloxuric bases from the urine, the fluid is supersaturated with ammonia and precipitated by a silver-nitrate solution. The precipitate is then decomposed with sulphuretted hydrogen. The boiling-hot filtrate is evaporated to dryness and the dried residue treated with 3 per cent sulphuric acid. The purine bases are dissolved, while the uric acid remains undissolved. This filtrate is saturated with ammonia and precipitated by silver-nitrate solution. If instead of precipitating with silver solution we desire to precipitate, according to Krüger and Wulff, with copper suboxide, the urine may be heated to boiling and immediately are added, successively, 100 c.c. of a 50 per cent sodium-bisulphate solution and 100 c.c. of a 12 per cent copper-sulphate solution for every liter of urine. The thoroughly washed precipitate is decomposed with hydrochloric acid and sulphuretted hydrogen. The uric acid remains in great part on the filter. Further details in regard to the treatment of the solution of the hydrochloric-acid compounds may be found in KRÜGER and SALOMON.[1]

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[1] Thudichum, "Grundzüge d. anal. med. klin. Chemie" (Berlin, 1886); Salomon, Arch. f. (Anat. u.) Physiol., 1882, and Ber. d. deutsch. chem. Gesellsch., **16** and **18**.

[2] Balke, "Zur Kenntniss der Xanthinkörper" (Inaug.-Diss., Leipzig, 1893); Salomon, Zeitschr. f. physiol. Chem., **18**.

[3] Arch. f. (Anat. u.) Physiol., 1894; Krüger and Salomon, Zeitschr. f. physiol. Chem., **24** and **26**.

Quantitative Estimation of Alloxuric Bases according to SALKOWSKI.[2] 400 to 600 c.c. of the urine free from protein is first precipitated by magnesia mixture and then by a 3 per cent silver-nitrate solution as described on page 577. The thoroughly washed silver preicpitate is decomposed by sulphuretted hydrogen after being suspended in 600–800 c.c. of water with the addition of a few drops of hydrochloric acid. It is heated to boiling and filtered hot, and finally evaporated to dryness on the water-bath. The residue is extracted with 20–30 c.c. of hot 3 per cent sulphuric acid and allowed to stand twenty-four hours; the uric acid is filtered off, washed, the filtrate made ammoniacal, and the xanthine bodies precipitated again by silver nitrate, the precipitate collected on a small chlorine-free filter, washed thoroughly, dried, carefully incinerated, the ash dissolved in nitric acid, and titrated with ammonium sulphocyanide according to VOLHARD's method. The ammonium-sulphocyanide solution should contain 1.2–1.4 grams per liter, and its strength should be determined by a silver-nitrate solution: 1 part silver corresponds to 0.277 gram nitrogen of alloxuric bases or to 0.7381 gram alloxuric bases. By this method the uric-acid and alloxuric bases can be simultaneously determined in the same portion of urine.[3]

MALFATTI[4] determines the nitrogen of the alloxuric bases in the hydrochloric-acid filtrate from the separated uric acid. This filtrate is evaporated with magnesia until all the ammonia has been expelled and the residue used for the KJELDAHL determination.

The nitrogen of the alloxuric bases is also determined as the difference between the uric-acid nitrogen and the total nitrogen of the alloxuric bodies of the silver precipitate (CAMERER, ARNSTEIN[5]). SALKOWSKI has raised the objection to this procedure that it is not possible to remove all the ammonia from the silver precipitate by washing. According to ARNSTEIN[6] this can readily be done by boiling the precipitate in water with some magnesia, and under these circumstances this method is quite serviceable. The nitrogen is estimated by KJELDAHL's method. The uric-acid nitrogen multiplied by 3 gives the quantity of uric acid. As the mixture of alloxuric bases in the urine is but little known, the quantity of nitrogen of the alloxuric bases is always calculated as a certain alloxuric base, for example xanthine (CAMERER), and the quantity so found used as a measure for the alloxuric bases.

According to a new method of KRÜGER and SCHMID[7] the uric acid and the purine bases are precipitated as a cuprous compound by copper-sulphate solution and sodium bisulphite. The precipitate is decomposed in sufficient water by sodium sulphide, and the uric acid precipitated from the concentrated filtrate with hydrochloric acid, and the purine bases again precipitated from this filtrate as cuprous or silver compounds. Finally, the nitrogen in the uric-acid part and the part containing the mixture of purine bases is estimated.

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[1] Zeitschr. f. physiol. Chem., **26**, and also Hoppe-Seyler-Thierfelder's Handbuch, 7, Aufl., 154.

[2] Pflüger's Arch., **69**.

[3] In regard to the details we refer the reader to the original paper.

[4] Centralbl. f. innere Med., 1897.

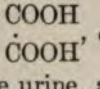
[5] Camerer, Zeitschr. f. Biologie, **26** and **28**; Arnstein, Zeitschr. f. physiol. Chem., **23**.

[6] Salkowski, l. c.; Arnstein, Centralbl. f. d. med. Wissensch., 1898.

[7] Zeitschr. f. physiol. Chem., **45** and Hoppe-Seyler-Thierfelder's Handbuch, 7. Aufl., 435.

We cannot discuss the other methods, such as those of Denigès and Niemilowicz, and the method suggested by Hall[1] for clinical purposes.

Oxaluric Acid, $C_3H_4N_2O_4=(CON_2H_3).CO.COOH$. This acid, whose relation to uric acid and urea has been spoken of above, does not always occur in the urine, and then only in traces as the ammonium salt. This salt is not directly precipitated by $CaCl_2$ and NH_3, but on boiling it is decomposed into urea and oxalate. In preparing oxaluric acid from urine the latter is filtered through animal charcoal. The oxalurate retained by the charcoal may be obtained by boiling with alcohol.

Oxalic Acid, $C_2H_2O_4$, or  $\begin{matrix}COOH\\ \cdot\\ COOH\end{matrix}$, occurs under physiological conditions in very small amounts in the urine, about 0.02 gram in twenty-four hours (Fürbringer[2]). According to the generally accepted view it exists in the urine as calcium oxalate, which is kept in solution by the acid phosphates present. Calcium oxalate is a frequent constituent of urinary sediments and occurs also in certain urinary calculi.

The origin of the oxalic acid in the urine is not well known. Oxalic acid when administered is eliminated unchanged, at least in part, by the urine;[3] and as many vegetables and fruits, such as cabbage, spinach, asparagus, sorrel, apples, grapes, etc., contain oxalic acid, it is possible that a part of the oxalic acid of the urine originates directly from the food. That oxalic acid may be formed in the animal body as a metabolic product from proteins or fats follows from the observations of Mills and Lüthje,[4] who found in dogs on an exclusively meat and fat diet, as also in starvation, that oxalic acid was eliminated by the urine. The oxalic acid which is eliminated in increased quantity with a diminished oxygen supply and an increased protein catabolism, as found by Reale and Boeri, and also by Terray, is supposed to be derived partly from the greater destruction of proteins. Pure protein does not, according to Salkowski,[5] increase the quantity of oxalic acid eliminated; on the contrary, after meat feeding the amount of this acid is increased, due in part to the meat containing oxalic acid (Salkowski). Gelatine and gelatine-yielding tissues seem to increase the excretion of oxalic acid, which stands in accord with the observations of Kutscher and Schenk[6] that on the oxidation of gelatine oxamic acid is produced from the glycocoll and this then decomposes readily into ammonia and oxalic acid. After feeding nucleins no constant increase in the elimination of oxalic acid has been observed.[1] The production of oxalic acid due to an incomplete combustion of the carbohydrates has also been suggested. The work of Hildebrandt and P. Mayer seems to indicate this under abnormal conditions. In alimentary glycosuria or diabetes Luzzato[2] could not observe any rise in the elimination of oxalic acid. We have no grounds for the assumption that oxalic acid is produced under physiological conditions by an incomplete combustion of carbohydrates. We cannot exclude the possibility of the formation of oxalic acid from the oxidation of uric acid in the animal body, yet we have no positive proof of such a formation.[3]

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[1] Niemilowicz, Zeitschr. f. physiol. Chem., **35**; Gittelmacher-Wilenko, *ibid.*, **36**; Hall, Wien. klin. Wochenschr., **16**.

[2] Deutsch. Arch. f. klin. Med., **18**. See also Dunlop, Journ. Path. and Bacteriol., **3**.

[3] In regard to the behavior of oxalic acid in the animal body, see pages 629 and 630.

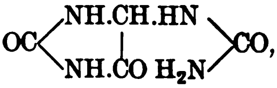
[4] Mills, Virchow's Arch., **99**; Lüthje, Zeitschr. f. klin. Med., **35**.

[5] Reale and Boeri, Wien. med. Wochenschr., 1895; Terray, Pflüger's Arch., **65**; Salkowski, Berl. klin. Wochenschr., 1900.

[6] Zeitschr. f. physiol. Chem., **43**.

Oxalic acid is best detected and quantitatively determined according to the method suggested by Salkowski: Shaking out the oxalic acid from the acidified urine by means of ether and then proceeding as follows according to Autenrieth and Barth:[4]

The twenty-four-hour urine is precipitated by $CaCl_2$ and ammonia in excess. After 18–20 hours the precipitate is collected (the filtrate must be clear) and dissolved in a little hydrochloric acid and shaken out 4–5 times with 150–200 c.c. ether (containing 3 per cent absolute alcohol). The united ethereal extracts are filtered through a dry filter and distilled after the addition of about 5 c.c. of water. The liquid, if necessary, is decolorized with animal charcoal and precipitated with $CaCl_2$ and ammonia, made acid after a certain time with acetic acid, and finally the oxalate is collected, washed, burned to CaO, and weighed.

Allantoin (Glyoxyldiureide), $C_4H_6N_4O_3$,  $OC\begin{matrix}\diagup NH.CH.HN\diagdown \\ \diagdown NH.\overset{|}{C}O\ H_2N\diagup\end{matrix}CO$, occurs in the urine of children within the first eight days after birth, and in very small amounts also in the urine of adults (Gusserow, Ziegler and Hermann). It is found in rather abundant quantities in the urine of pregnant women (Gusserow). Allantoin has also been found in the urine of sucking calves (Wöhler), in urine of oxen (Salkowski), and sometimes in the urine of other animals (Meissner). It is also found in the amniotic fluid and, as first shown by Vauquelin and Lassaigne[5] in the allantoic fluid of the cow (hence the name). Allantoin is formed, as above stated, by the oxidation of uric acid outside of the animal body, hence a similar formation of allantoin is assumed in the animal organism (see page 573). According to PODUSCHKA and MINKOWSKI,[1] allantoin introduced into dogs appears almost entirely in the urine, while in man only a small portion of the ingested substance is eliminated by the kidneys. In carnivora the excretion of allantoin is considerably increased, according to MINKOWSKI, COHN, SALKOWSKI, and MENDEL and BROWN,[2] after feeding thymus or pancreas. According to MENDEL and WHITE, on the intravenous injection of urates an abundant elimination of allantoin takes place in dogs and cats. A strong allantoin excretion is also found in dogs after poisoning with hydrazine (BORISSOW), hydroxylamine, semicarbazide, and aminoguanidine (POHL [3]). He also obtained allantoin in the autolysis of the intestinal mucosa, liver, thymus, spleen, and pancreas. As no allantoin exists in the organs of normal starving dogs, and as POHL has found it in the liver and, as traces, also in the other organs after poisoning with hydrazine, he claims that the allantoin is formed in the nuclein destruction produced by the death of the cell-nuclei.

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[1] See Stradomsky, Virchow's Arch., **163**; Mohr and Salomon, Deutsch. Arch. f. klin. Med., **70**; Salkowski, l. c.

[2] Hildebrandt, Zeitschr. f. physiol. Chem., **35**; P. Mayer, Zeitschr. f. klin. Med., **47**; Luzzato, Salkowski's Festschrift, 1904.

[3] See Wiener, Ergebnisse der Physiol., **1**, Abt. 1.

[4] Salkowski, Zeitschr. f. physiol. Chem., **29**; Autenrieth and Barth, *ibid.*, **35**.

[5] Ziegler and Hermann, see Gusserow, Arch. f. Gynäkol, **3**—both cited from Huppert-Neubauer, Harn-Analyse, 10. Aufl., 377; Wöhler, Annal. d. Chem. u. Pharm., **70**; Salkowski, Zeitschr. f. physiol. Chem., **42**; Meissner, Zeitschr. f. rat. Med. (3), **31**; Lassaigne, Annal. de Chim. et Phys., **17**.

Allantoin is a colorless substance often crystallizing in prisms, difficultly soluble in cold water, easily soluble in boiling water, and also in warm alcohol, but not soluble in cold alcohol or ether. A watery allantoin solution gives no precipitate with silver nitrate alone, but by the careful addition of ammonia a white flocculent precipitate is formed, $C_4H_5AgN_4O_3$, which is soluble in an excess of ammonia and which consists after a certain time of very small, transparent microscopic globules. The dry precipitate contains 40.75 per cent silver. A watery allantoin solution is precipitated by mercuric nitrate. On continued boiling allantoin reduces FEHLING'S solution. It gives SCHIFF'S furfurol reaction less rapidly and less intensely than urea. Allantoin does not give the murexide test.

Allantoin is most easily prepared by the oxidation of uric acid with lead peroxide. In preparing allantoin from urine, proceed according to LOEWY'S [4] method, which consists of the following: The faintly acidified urine is precipitated with a mercurous-nitrate solution, the filtrate treated with H_2S, and the new filtrate precipitated by magnesium oxide and silver nitrate after the removal of the H_2S. The precipitate is filtered off and washed with warm water and decomposed with H_2S, and the filtrate evaporated to dryness. The residue is extracted with hot water and then the solution precipitated with mercuric nitrate. The precipitate is collected and decomposed by H_2S. From the evaporated filtrate the allantoin crystallizes out. This method can be used for the quantitative determination of allantoin.

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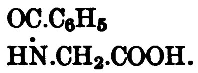
[1] Arch. f. exp. Path. u. Pharm., **44**; Minkowski, *ibid.*, **41**.

[2] Minkowski, l. c., and Centralbl. f. innere Med., 1898; Cohn, Zeitschr. f. physiol. Chem., **25**; Salkowski, Centralbl. f. d. med. Wissensch., 1898; Mendel and Brown, Amer. Journ. of Physiol., **3**.

[3] Mendel and White, Amer. Journ. of Physiol., **12**; Borissow, Zeitschr. f. physiol. Chem., **19**; Pohl, Arch. f. exp. Path. u. Pharm., **46**.

[4] Arch. f. exp. Path. u. Pharm., **44**.

Hippuric Acid (Benzoyl-amino acetic acid), $C_9H_9NO_3$,



$$\begin{array}{l} OC.C_6H_5 \\ HN.CH_2.COOH. \end{array}$$

This acid decomposes into benzoic acid and glycocoll on boiling with mineral acids or alkalies, and also by the putrefaction of the urine. The reverse of this occurs if these two components are heated in a sealed tube, according to the following equation: $C_6H_5COOH + NH_2.CH_2.COOH = C_6H_5.CO.NH.CH_2.COOH + H_2O$. This acid may be synthetically prepared from benzamide and monochloracetic acid, $C_6H_5.CO.NH_2 + CH_2Cl.COOH = C_6H_5.CO.NH.CH_2.COOH + HCl$, and in various other ways, but most simply from glycocoll and benzoyl chloride in the presence of alkali.

Hippuric acid occurs in large amounts in the urine of herbivora, but only in small quantities in that of carnivora. The quantity of hippuric acid eliminated in human urine on a mixed diet is usually less than 1 gram per day; as an average it is 0.7 gram. After eating freely of vegetables and fruit, especially such fruit as plums, the quantity may be more than 2 grams. Hippuric acid is also found in the perspiration, the blood, the suprarenal capsule of oxen, and in ichthyosis scales. Nothing is positively known in regard to the quantity of hippuric acid in the urine in disease.

The *Formation of Hippuric Acid* in the Organism. Benzoic acid and also the substituted benzoic acids are converted into hippuric acid and substituted hippuric acids within the body. Moreover, those bodies are transformed into hippuric acid which by oxidation (toluene, cinnamic acid, hydrocinnamic acid) or by reduction (quinic acid) are converted into benzoic acid. The question of the origin of hippuric acid is therefore connected with the question of the origin of benzoic acid; the formation of the second component, glycocoll, from the protein substances in the body is unquestionable.

Hippuric acid is found in the urine of starving dogs (Salkowski), also in dog's urine after a diet consisting entirely of meat (Meissner and Shepard, Salkowski, and others[1]). It is evident that the benzoic acid originates in these cases from the proteins, and it is generally admitted that it is produced by the putrefaction of proteins in the intestine. Among the products of the putrefaction of protein outside of the body Salkowski has found phenylpropionic acid, $C_6H_5.CH_2.CH_2.COOH$, which is oxidized in the organism to benzoic acid and eliminated as hippuric acid after combining with glycocoll. Phenylpropionic acid seems to be formed from the aminophenylpropionic acid, which is derived from several protein substances. The supposition that the phenylpropionic acid is produced from tyrosine by putrefaction in the intestine has not been substantiated by the researches of BAUMANN, SCHOTTEN, and BAAS.[1] The importance of putrefaction in the intestine in producing hippuric acid is evident from the fact that after thoroughly disinfecting the intestine of dogs with calomel the hippuric acid disappears from the urine (BAUMANN [2]).

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[1] Salkowski, Ber. d. deutsch. chem. Gesellsch., 11; Meissner and Shepard, Untersuch. über das Entstehen der Hippursäure im thierischen Organismus. Hanover, 1866.

The large quantity of hippuric acid present in the urine of herbivora is partly explained by the specially active processes of putrefaction going on in the intestines of these animals, but it is especially due to the large quantity of substances in the plant-food from which benzoic acid can be formed. There is hardly any doubt that the hippuric acid in human urine after a mixed diet, and especially after a diet of vegetables and fruits, originates in part from the aromatic substances, e.g., quinic acid.

The view proposed by WEISS and others that a parallelism exists between the excretion of hippuric acid and uric acid in that an increase in the first is followed by a diminution in the second, and that, for example, quinic acid produces a diminution in the excretion of uric acid corresponding to the increased formation of hippuric acid (WEISS, LEWIN), cannot be considered as sufficiently proved [3] (HUPFER).

The kidneys may be considered in dogs as special organs for the synthesis of hippuric acid (SCHMIEDEBERG and BUNGE [4]). In other animals as in rabbits, the formation of hippuric acid seems to take place in other organs, such as the liver and muscles. The synthesis of hippuric acid is therefore not exclusively limited to any special organ, though perhaps in some species of animals it may be more abundant in one organ than in another.

As the thorough investigations of WIECHOWSKI teach, the synthesis of hippuric acid does not stand in any direct relationship to the extent of protein metabolism; it varies, on the contrary, with the duration of circulation of benzoic acid and the quantity of glycocoll present in the body. The amount of the latter in intermediary metabolism is so great that in rabbits, on the administration of benzoic acid, more than one half of the total urine nitrogen may exist as glycocoll. MAGNUS-LEVY [5] found in rabbits and sheep up to 27.8 per cent of the total nitrogen as hippuric-acid nitrogen, and both investigators have found so much hippuric-acid nitrogen that it could not be accounted for by the glycocoll preformed from the proteins. We cannot explain how this abundant formation and elimination of glycocoll as hippuric acid is brought about.

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[1] E. and H. Salkowski, Ber. d. deutsch. chem. Gesellsch., **12**; Baumann, Zeitschr. f. physiol. Chem., **7**; Schotten, *ibid.*, 8; Baas, *ibid.*, **11**.

[2] *Ibid.*, **10**, 131.

[3] Weiss, Zeitschr. f. physiol. Chem., **25**, **27**, **38**; Lewin, Zeitschr. f. klin. Med., **42**; Hupfer, Zeitschr. f. physiol. Chem., **37**. See also Wiener, "Die Harnsäure," Ergebnisse der Physiol., **1**, Abt. 1.

[4] Arch. f. exp. Path. u. Pharm., **6**; also A. Hoffmann, *ibid.*, **7**, and Kochs, Pflüger's Arch., **20**; Bashford and Cramer, Zeitschr. f. physiol. Chem., **35**.

[5] Wiechowski, Hofmeister's Beiträge, **7** (literature); A. Magnus-Levy, Münch. med. Wochenschr., 1905.

Properties and Reactions of Hippuric Acid. This acid crystallizes in semi-transparent, long, four-sided, milk-white, rhombic prisms or columns, or in needles by rapid crystallization. They dissolve in 600 parts cold water, but more easily in hot water. They are easily soluble in alcohol, but with difficulty in ether. The acid dissolves more easily (about 12 times) in acetic ether than in ethyl ether. Petroleum-ether does not dissolve hippuric acid.

On heating hippuric acid it first melts at 187.5° C. to an oily liquid which crystallizes on cooling. On continuing to heat it decomposes, producing a red mass and a sublimate of benzoic acid, with the generation, first, of a peculiar pleasant odor of hay and then an odor of hydrocyanic acid. Hippuric acid is easily differentiated from benzoic acid by this behavior, also by its crystalline form and its insolubility in petroleum ether. Hippuric acid and benzoic acid both give LÜCKE's reaction, namely, they generate an intense odor of nitrobenzene when evaporated to dryness with nitric acid and when the residue is heated with sand in a glass tube. Hippuric acid forms crystallizable salts, in most cases, with bases. The combinations with alkalies and alkaline earths are soluble in water and alcohol. The silver, copper, and lead salts are soluble with difficulty in water; the ferric salt is insoluble.

Hippuric acid is best prepared from the fresh urine of a horse or cow. The urine is boiled a few minutes with an excess of milk of lime. The liquid is filtered while hot, concentrated and then cooled, and the hippuric acid precipitated by the addition of an excess of hydrochloric acid. The crystals are pressed, dissolved in milk of lime by boiling, and treated as above; the hippuric acid is precipitated again from the concentrated filtrate by hydrochloric acid. The crystals are purified by recrystallization and decolorized, when necessary, by animal charcoal.

The quantitative estimation of hippuric acid in the urine may be performed by the following method (BUNGE and SCHMIEDEBERG[1]): The urine is first made faintly alkaline with soda, evaporated nearly to dryness, and the residue thoroughly extracted with strong alcohol. After the evaporation of the alcohol the residue is dissolved in water, the solution acidified with sulphuric acid, and completely extracted by agitating (at least five times) with fresh portions of acetic ether. The acetic ether is then repeatedly washed with water, which is removed by means of a separatory funnel, then evaporated at a medium temperature and the dry residue treated repeatedly with petroleum-ether, which dissolves the benzoic acid, oxyacids, fats, and phenols, while the hippuric acid remains undissolved. This residue is now dissolved in a little warm water and evaporated at 50–60° C. to crystallization. The crystals are collected on a small weighed filter. The mother-liquor is repeatedly shaken with acetic ether. This last is removed and evaporated; the residue is added to the above crystals on the filter, dried and weighed.

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[1] Arch. f. exp. Path. u. Pharm., 6. In regard to other methods, such as Blumenthal as well as Pfeiffer, Bloch and Riecke, see Maly's Jahresber., **30** and **32**. See also Wiechowski, l. c.

Phenaceturic Acid, $C_{10}H_{11}NO_3 = C_6H_5.CH_2.CO.NH.CH_2.COOH$. This acid, which is produced in the animal body by a combination of glycocoll with the phenylacetic acid, $C_6H_5.CH_2.COOH$, formed in the putrefaction of the proteins, has been prepared from horse's urine by SALKOWSKI,[1] but it probably also occurs in human urine.

Benzoic Acid, $C_7H_6O_2$ or $C_6H_5.COOH$, is found in rabbit's urine and sometimes, though in small amounts, in dog's urine (WEYL and v. ANREP). According to JAARSVELD and STOKVIS and to KRONECKER it is also found in human urine in diseases of the kidneys. The occurrence of benzoic acid in the urine seems to be due to a fermentative decomposition of hippuric acid. Such a decomposition may very easily occur in an alkaline urine or in one containing proteid (VAN DE VELDE and STOKVIS). In certain animals—pigs and dogs—the kidneys, according to SCHMIEDEBERG and MINKOWSKI,[2] contain a special enzyme, SCHMIEDEBERG'S *histozym*, which splits the hippuric acid with the separation of benzoic acid.

Ethereal Sulphuric Acids. In the putrefaction of proteins in the intestine, phenols, whose mother-substance is considered to be tyrosine, and also indol and skatol are produced. These phenols directly, and the two last-named bodies after they have been oxidized respectively into indoxyl and skatoxyl, pass into the urine as ethereal sulphuric acids after uniting with sulphuric acid. The most important of these ethereal acids are *phenol-* and *cresol-sulphuric acids*—which were formerly also called phenol-forming substances—*indoxyl-* and *skatoxyl-sulphuric acids.* To this group belong also the *pyrocatechin-sulphuric acid,* which occurs only in very small amounts in human urine, and *hydroquinone-sulphuric acid,* which appears in the urine after poisoning with phenol, and under physiological conditions perhaps other ethereal acids occur which have not been isolated. The ethereal sulphuric acids of the urine were discovered and specially studied by BAUMANN.[3] The quantity of these acids in human urine is small, while horse's urine contains larger quantities. According to the determinations of v. D. VELDEN the quantity of ethereal sulphuric acid in human urine in twenty-four hours varies between 0.094 and 0.620 gram. The relationship of the sulphate-sulphuric acid *A* to the conjugated sulphuric acid *B* in health is on an average as 10:1. It undergoes such great variations, as found by Baumann and Herter,[1] and after them by many other investigators, that it is hardly possible to consider the average figures as normal. After taking phenol and certain other aromatic substances, as well as when putrefaction within the organism is general, the elimination of ethereal sulphuric acid is greatly increased. On the contrary, it is diminished when the putrefaction in the intestine is reduced or prevented. For this reason it may be greatly diminished by carbohydrates and exclusive milk diet.[2] The intestinal putrefaction and the elimination of ethereal sulphuric acid have also been diminished in some cases by certain therapeutic agents which have an antiseptic action; still the statements are not unanimous.[3]

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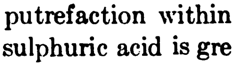
[1] Zeitschr. f. physiol. Chem., **9**.

[2] Weyl and v. Anrep, Zeitschr. f. physiol. Chem., **4**; Jaarsveld and Stokvis, Arch. f. exp. Path. u. Pharm., **10**; Kronecker, *ibid*, **16**; Van der Velde and Stokvis, *ibid.*, **17**; Schmiedeberg, *ibid.*, **14**, 379; Minkowski, *ibid.*, **17**.

[3] Pflüger's Arch., **12** and **13**.

Great importance has been given to the relationship between the total sulphuric acid and the conjugated sulphuric acid, or between the conjugated sulphuric acid and the sulphate-sulphuric acid, in the study of the intensity of the putrefaction in the intestine under different conditions. Several investigators, F. Müller, Salkowski, and v. Noorden,[4] consider correctly that this relationship is only of secondary value, and that it is more correct to consider the absolute value. It must be remarked that the absolute values for the conjugated sulphuric acid also undergo great variation, so that it is at present impossible to give the upper or lower limit for the normal value.



Phenol- and p-Cresol-sulphuric Acids, $C_6H_5.O.SO_2.OH$ and $C_6H_4 < \begin{matrix} O.SO_2.OH \\ CH_3 \end{matrix}$. These acids are found as alkali salts in human urine, in which also orthocresol has been detected. The quantity of cresol-sulphuric acid is considerably greater than of phenol-sulphuric acid. In the quantitative estimation the phenols are set free from the two ethereal acids and determined together as tribromphenol. The quantity of phenols which are separated from the ethereal-sulphuric acids of the urine amounts to 17–51 milligrams in the twenty-four hours (Munk). The methods for the quantitative estimation used heretofore give, according to Rumpf, as well as Kossler and Penny,[5] such inaccurate results that new determinations are very desirable. After a vegetable diet the quantity of these ethereal-sulphuric acids is greater than after a mixed diet. After the ingestion of carbolic acid, which is in great part converted by synthesis within the organism into phenol-sulphuric acid, also into pyrocatechin- and hydroquinon-sulphuric acid,[1] or when the amount of sulphuric acid is not sufficient to combine with the phenol, it forms phenyl-glucuronic acid,[2] the quantity of phenols and ethereal-sulphuric acids in the urine is considerably increased at the expense of the sulphate-sulphuric acid.

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[1] v. d. Velden, Virchow's Arch., 70; Herter, Zeitschr. f. physiol. Chem., 1.

[2] See Hirschler, Zeitschr. f. physiol. Chem., 10; Biernacki, Deutsch. Arch. f. klin. Med., 49; Rovighi, Zeitschr. f. physiol. Chem., 16; Winternitz, *ibid.*, and Schmitz, *ibid.*, 17 and 19.

[3] See Baumann and Morax, Zeitschr. f. physiol. Chem., 10; Steiff, Zeitschr. f. klin. Med., 16; Rovighi, l. c., Stern, Zeitschr. f. Hygiene, 12; and Bartoschewitsch, Zeitschr. f. physiol. Chem., 17; Mosse, *ibid.*, 23.

[4] Müller, Zeitschr. f. klin. Med., 12; v. Noorden, *ibid.*, 17; Salkowski, Zeitschr. f. physiol. Chem., 12.

[5] Munk, Pflüger's Arch., 12; Rumpf, Zeitschr. f. physiol. Chem., 16; Kossler and Penny, *ibid.*, 17.

An increased elimination of phenol-sulphuric acids occurs in active putrefaction in the intestine with stoppage of the contents of the intestine, as in ileus, diffused peritonitis with atony of the intestine, or tuberculous enteritis, but not in simple obstruction. The elimination is also increased by the absorption of the products of putrefaction from purulent wounds or abscesses. An increased elimination of phenol has been observed in a few other cases of diseased conditions of the body.[3]

The alkali salts of phenol- and cresol-sulphuric acids crystallize in white plates, similar to mother-of-pearl, which are rather freely soluble in water. They are soluble in boiling alcohol, but only slightly soluble in cold alcohol. On boiling with dilute mineral acids they are decomposed into sulphuric acid and the corresponding phenol.

Phenol-sulphuric acids have been synthetically prepared by BAUMANN from potassium pyrosulphate and potassium phenolate or *p*-cresolate. For the method of their preparation from urine, which is rather complicated, and also for the known phenol reactions, the reader is referred to other text-books. The quantitative estimation of these ethereal-sulphuric acids was usually performed by weighing the phenol which was separated from the urine as tribromphenol. At the present time the following method is employed:

KOSSLER and PENNY'S Method with NEUBERG'S[4] Modification. The liquid containing phenol is treated with N/10 caustic soda until strongly alkaline, warmed on the water-bath in a flask with a glass stopper, and then treated with an excess of N/10 iodine solution, the quantity being exactly measured. Sodium iodide is first formed and then sodium hypoiodite, which latter forms tri-iodophenol with the phenol according to the following equation:

$$C_6H_5OH + 3NaIO = C_6H_2I_3.OH + 3NaOH.$$

On cooling acidify with sulphuric acid and determine the excess of iodine by titration with N/10 sodium thiosulphate solution. This process is also available for the estimation of paracresol. Each cubic centimeter of the iodine solution used is equivalent to 1.5670 milligrams of phenol or 1.8018 milligrams of cresol. As the determination does not give any idea as to the variable proportions of the two phenols, the quantity of iodine used must be calculated as one or the other of the two phenols. Before such a determination is carried out, the concentrated urine is first distilled after acidification with sulphuric acid and the distillate purified by precipitation with lead and distilled again (NEUBERG). For details, see NEUBERG, l. c., and HOPPE-SEYLER-THIERFELDER'S Handbuch, 7. Aufl.

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[1] See Baumann, Pflüger's Arch., **12** and **13**, and Baumann and Preusse, Zeitschr. f. physiol. Chem., **3**, 156.

[2] Schmiedeberg, Arch. f. exp. Path. u. Pharm., **14**.

[3] See G. Hoppe-Seyler, Zeitschr. f. physiol. Chem., **12**, (this contains also all references to the literature on this subject); Fedeli, Moleschott's Untersuch., **15**.

[4] Kossler and Penny, Zeitschr. f. physiol. Chem., **17**; Neuberg, *ibid.*, **27**.

The methods for the separate determination of the conjugated sulphuric acid and the sulphate-sulphuric acid will be spoken of later in connection with the determination of the sulphuric acid of the urine.

Pyrocatechin-sulphuric Acid. This acid was first found in horse's urine in rather large quantities by BAUMANN. It occurs in human urine only in the very smallest amounts, and perhaps not constantly, but it is present abundantly in the urine after taking phenol, pyrocatechin, or protocatechuic acid.

With an exclusively meat diet this acid does not occur in the urine, and it therefore must originate from vegetable food. It probably originates from the protocatechuic acid, which, according to PREUSSE, passes in part into the urine as pyrocatechin-sulphuric acid. This acid may also perhaps be formed by the oxidation of phenol within the organism (BAUMANN and PREUSSE [1]).

Pyrocatechin, or O-DIOXYBENZENE, $C_6H_4(OH)_2$, was first observed in the urine of a child (EBSTEIN and J. MÜLLER). The reducing body ALCAPTON, first found by BÖDEKER [2] in human urine and which was considered for a long time as identical with pyrocatechin, is in most cases probably *homogentisic acid* or *uroleucic acid* (see below).

Pyrocatechin crystallizes in prisms which are soluble in alcohol, ether, and water. It melts at 102–104° C., and sublimes in shining plates. The watery solution becomes green, brown, and ultimately black in the presence of alkali and the oxygen of the air. If very dilute ferric chloride is treated with tartaric acid and then made alkaline with ammonia, and this added to a watery solution of pyrocatechin, we obtain a violet or cherry-red liquid which becomes green on adding excess of acetic acid. Pyrocatechin is precipitated by lead acetate. It reduces an ammoniacal silver solution at the ordinary temperature, and reduces alkaline copper-oxide solutions with heat, but does not reduce bismuth oxide.

A urine containing pyrocatechin, if exposed to the air, especially when alkaline, quickly becomes dark and reduces alkaline copper solutions when heated. In detecting pyrocatechin in the urine it is concentrated when necessary, filtered, boiled with the addition of sulphuric acid to remove the phenols, and repeatedly shaken after cooling with ether. The ether is distilled from the several ethereal extracts, the residue neutralized with barium carbonate and shaken again with ether. The pyrocatechin which remains after evaporating the ether may be purified by recrystallization from benzene.

Hydroquinone, or P-DIOXYBENZENE, $C_6H_4(OH)_2$, often occurs in the urine after the use of phenol (BAUMANN and PREUSSE). The dark color which certain urines, so-called "carbolic urines," assume in the air is due to decomposition products. Hydroquinone does not occur as a normal constituent of urine, but only after the administration of hydroquinone; and according to LEWIN,[3] it may be found in the urine of rabbits as an ethereal-sulphuric acid, being a decomposition product of arbutin.

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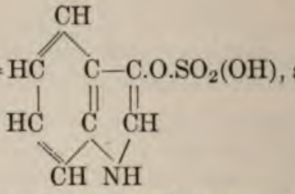
[1] Baumann and Herter, Zeitschr. f. physiol. Chem., 1; Preusse, *ibid.*, 2; Baumann, *ibid.*, 3.

[2] Ebstein and Müller, Virchow's Arch., 62; Bödeker, Zeitschr. f. rat. Med. (3), 7.

[3] Virchow's Arch., 92.

Hydroquinone forms rhombic crystals which are readily soluble in water, alcohol, and ether. It melts at 169° C. Like pyrocatechin, it easily reduces metallic oxides. It acts like pyrocatechin with alkalies, but is not precipitated with lead acetate. It is oxidized into quinone by ferric chloride and other oxidizing agents, and quinone can be detected by its peculiar odor. Hydroquinone-sulphuric acid is detected in the urine by the same methods as pyrocatechin-sulphuric acid.

Indoxyl-sulphuric Acid, $C_8H_7NSO_4$ =



also called URINE INDICAN, formerly called UROXANTHINE (HELLER), occurs as an alkali-salt in the urine. This acid is the mother-substance of a great part of the indigo of the urine. The quantity of indigo which can be separated from the urine is considered as a measure of the quantity of indoxyl-sulphuric acid (and indoxyl-glucuronic acid) contained in the urine. This amount, according to JAFFÉ,[1] for man is 5–20 milligrams per twenty-four hours. Horse's urine contains about twenty-five times as much indigo-forming substance as human urine.

Indoxyl-sulphuric arid is derived, as previously mentioned (page 401), from indol, which is first oxidized in the body into indoxyl and is then coujugated with sulphuric acid. After subcutaneous injection of indol the elimination of indican is considerably increased (JAFFÉ, BAUMANN and BRIEGER, and others). It is also increased by the introduction of orthonitrophenylpropiolic acid in the animal organism (G. HOPPE-SEYLER[2]) Indol is formed by the putrefaction of proteins. The putrefaction of secretions rich in protein in the intestine explains also the occurrence of indican in the urine during starvation. Gelatine, on the contrary, does not increase the elimination of indican.

An abnormally increased elimination of indican occurs in those diseases where the small intestines are obstructed, causing an increased putrefaction and thus producing an abundance of indol. Such an increased elimination of indican occurs on tying the small intestine of a dog, but not the large intestine (JAFFÉ), an observation which has been confirmed recently by ELLINGER and PRUTZ.[3] They removed an intestinal loop in dogs and replaced it in a reversed position, the distal end of the loop being attached to the proximal end of the intestine, and in this manner, by the inverted peristalsis so obtained, they effected a disturbance in the movement of the intestinal contents. It was shown that this obstruction in the small intestine caused an increased elimination of indican, while an obstruction in the large intestine showed no such action.

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[1] Pflüger's Arch., **3**.

[2] Jaffé, Centralbl. f. d. med. Wissensch., 1872; Baumann and Brieger, Zeitschr. f. physiol. Chem., **3**; G. Hoppe-Seyler, *ibid.*, **7** and **8**. See also Porcher and Hervieux, Journ. de Physiol., **7**.

[3] Jaffé, Virchow's Arch., **70**; Ellinger and Prutz, Zeitschr. f. physiol. Chem., **38**.

The putrefaction of proteins in other organs and tissues besides the intestine may also cause an increase in the indican of the urine. Certain investigators, BLUMENTHAL, ROSENFELD, and LEWIN, claim to have shown that an increased excretion of indican can be brought about also without putrefaction by an increased destruction of tissue in starvation and also after phlorhizin poisoning; but these statements are vehemently opposed by other investigators, such as P. MAYER, SCHOLZ, and ELLINGER, and are improbable. The indol, it seems, is not formed from the tryptophane (indolaminopropionic acid) as intermediary step in the demolition of the proteins in the animal body, but rather from the putrefaction of the tryptophane in the intestine. GENTZEN[1] has also shown that tryptophane introduced subcutaneously or per os into the body does not lead to an indicanuria, but only when it is exposed to bacterial decomposition in the large intestine. The statements as to the elimination of indican after oxalic-acid poisoning are somewhat contradictory. After poisoning with oxalic acid HARNACK and v. LEYEN found an increased indican elimination, and MORACZEWSKI believes he has proved a certain parallelism between the quantity of indican and the quantity of oxalic acid in diabetes. SCHOLZ[2] obtained, on the contrary, no increase in the excretion of indican after oxalic-acid poisoning.

An increased elimination of indican has been observed in many diseases,[3] and in these cases the quantity of phenol eliminated is also generally increased. A urine rich in phenol is not always rich in indican.

The potassium salt of indoxyl-sulphuric acid, which was prepared pure by BAUMANN and BRIEGER from the urine of a dog fed on indol, has since been prepared synthetically by BAUMANN and THESEN,[4] by fusing phenylglycine-orthocarboxylic acid with alkali and then from this producing the indoxylsulphate by means of potassium pyrosulphate. It crystallizes in colorless, shining plates or leaves which are easily soluble in water, but less readily in alcohol. It is split by mineral acids into sulphuric acid and indoxyl. The latter without access of air passes into a red compound, indoxyl-red, but in the presence of oxidizing reagents is converted into indigo-blue: $2C_8H_7NO+2O=C_{16}H_{10}N_2O_2+2H_2O$. The detection of indican is based on this last fact.

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[1] Blumenthal, Arch. f. (Anat. u.) Physiol., 1901, Suppl., and 1902, with Rosenfeld, Charité annalen, 27. and Hofmeister's Beiträge, 5; Lewin, Hofmeister's Beiträge, 1; Mayer, Arch. f. (Anat. u.) Physiol., 1902. Zeitschr. f. klin. Med., 47, and Zeitschr. f. physiol. Chem., 29, 32; Scholz, *ibid.*, 38; Ellinger, *ibid.*, 39; Gentzen, "Über die Vorstufen des Indols bei der Eiweissfäulnis im Thierkörper," Inaug.-Dissert. Köngisberg, 1904.

[2] Harnack, Zeitschr. f. physiol. chemie, 29; Scholz, l. c.; Moraczewski, Centralbl. f. innere Med., 1903.

[3] See Jaffé, Pflüger's Arch., 3; Senator, Centralbl. f. d. med. Wissensch., 1877; G. Hoppe-Seyler, Zeitschr. f. physiol. Chem., 12 (contains older literature); also Berl. klin. Wochenschr., 1892.

[4] Baumann with Brieger, Zeitschr. f. physiol. Chem., 3; with Thesen, *ibid.*, 23.

For the rather complicated preparation of indoxyl-sulphuric acid as the potassium salt from urine the reader is referred to other text-books. For the detection of indican in urine in ordinary cases the following method of JAFFÉ-OBERMAYER, which also serves as an approximate test for the quantity of indican, is sufficient.

JAFFÉ-OBERMAYER'S *Indican Test.* JAFFÉ uses chloride of lime as the oxidizing agent, while OBERMAYER employs ferric chloride. Other oxidizing agents have been suggested, such as potassium permanganate, potassium bichromate, alkali chlorate, and hydrogen peroxide (the latter suggested by PORCHER and HERVIEUX[1]). With OBERMAYER'S reagent the test is performed as follows:

The acid urine (if alkaline it must be acidified with acetic acid) (ELLINGER) is precipitated with basic lead acetate, 1 c.c. for every 10 c.c. of the urine. 20 c.c. of the filtrate are treated in a test-tube with an equal volume of pure concentrated hydrochloric acid (specific gravity 1.19) which contains 2–4 grams ferric chloride to the liter, and 2–3 c.c. chloroform are added and the mixture immediately thoroughly shaken. The chloroform is thereby colored more or less blue, depending upon the amount of indican. Besides indigo blue we may also have indigo red produced, whose formation has been explained in various ways. The quantity of indigo red becomes greater the more slowly the oxidation takes place, and especially when the decomposition takes place in the warmth (see the works of ROSIN, BOUMA, WANG, MAILLARD, and ELLINGER[2]).

According to ELLINGER the source of the indigo-red formation may be the isatin that is produced by the superoxidation of the indoxyl by the action of the reagent, and this isatin forms indigo red with the indoxyl in the hydrochloric-acid solution. MAILLARD, on the contrary, is of the view that the blue substance which is taken up by the chloroform from the urine mixed with hydrochloric acid is not indigotin (indigo-blue), but another substance, called by him hemi-indigotin, which in alkaline solution polymerizes immediately into indigotin, while in acid reaction it is converted into indirubin (indigo red).

The chloroform solution of indigo obtained in the indican test may be used in the quantitative colorimetric determination by comparison with a solution of indigo in chloroform of known strength (KRAUSS and ADRIAN).

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[1] Jaffé, Pflüger's Arch., **3**; Obermayer, Wien. klin. Wochenschr., 1890; Porcher and Hervieux, Zeitschr. f. physiol. Chem., **39**.

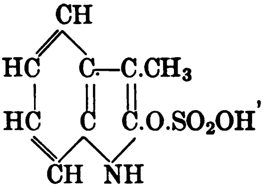
[2] Rosin, Virchow's Arch., **123**; Bouma, Zeitschr. f. physiol. Chem., **27**, **30**, **32**, **39**; Wang, *ibid.*, **25**, **27**, **28**; Ellinger, *ibid.*, **38** and **41**; Maillard, Bull. soc. chim., Paris (3), **29**, and Compt. rend., **136**; also L'indoxyle urinaire et les couleurs qui en derivent, Paris, 1903, and Zeitschr. f. physiol. Chem., **41**.

WANG and others convert the indigo into indigo-sulphonic acid by concentrated sulphuric acid and titrate with potassium permanganate. There is still doubt as to the surest and most trustworthy method for the determination of indican, and especially as to the question how the indigo residue is to be washed (see WANG, BOUMA, ELLINGER, and SALKOWSKI [1]), and for this reason we shall refer only to the works cited above.

Because of the difficulty arising from the production of indirubin in addition to indigotin, BOUMA has recommended the conversion of all the indoxyl into indirubin by boiling the urine with hydrochloric acid containing isatin. The indirubin can be taken up by chloroform and determined by titration with potassium permanganate and sulphuric acid after purification of the chloroform residue. OERUM [2] has also worked out a colorimetric method of estimation based upon BOUMA's method.

Indol seems also to pass into the urine as a glucuronic acid, *indoxyl-glucuronic acid* (SCHMIEDEBERG). Such an acid has been found in the urine of animals after the administration of the sodium-salt of *o*-nitro-phenyl-propiolic acid (G. HOPPE-SEYLER). PORCHER and HERVIEUX [3] have obtained indoxyl sulphuric acid in dogs and asses under similar conditions.

Free indigo, and in fact indirubin as well as indigotin, occur in rare cases in the undecomposed urine. GRÖBER and WANG [4] have recently observed such cases.

Skatoxyl-sulphuric Acid, $C_9H_9NSO_4 =$ , has not been positively prepared as a constituent of normal urine, but OTTO has once prepared its alkali salt from diabetic urine. Perhaps skatoxyl occurs in normal urine as a conjugated glucuronate (MAYER and NEUBERG [5]), and it is believed that the urine contains a skatol-chromogen from which red and reddish-violet coloring-matters are obtained by decomposition with strong acids and an oxidizing agent.

Skatoxyl-sulphuric acid originates, if it exists in the urine, from skatol which is formed by putrefaction in the intestine, and which is then conjugated with sulphuric acid after oxidation into skatoxyl. That skatol introduced into the body passes partly as an ethereal-sulphuric acid into the urine has been shown by BRIEGER. Indol and skatol act differently, at least in dogs; indol producing a considerable amount of ethereal-sulphuric acid, while skatol gives only a small quantity (MESTER[1]). The statements are somewhat contradictory on this subject and the behavior is somewhat unsettled. According to STAAL the chromogen of the skatol red is neither a conjugated sulphuric acid nor a conjugated glucuronic acid.

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[1] Krauss, Zeitschr. f. physiol. Chem., 18; Adrian, *ibid.*, 19; Wang, *ibid.*, 25; Salkowski, *ibid.*, 42.

[2] Bouma, Zeitschr. f. physiol. Chem., 32; Oerum, *ibid.*, 45.

[3] Schmiedeberg, Arch. f. exp. Path. u. Pharm., 14; G. Hoppe-Seyler, Zeitschr. f. physiol. Chem., 7 and 8; Porcher and Hervieux, Journ. de Physiol., 7.

[4] Gröber, Münch. med. Wochenschr., 1904; Wang, Salkowski's Festschrift, 1904.

[5] Otto, Pflüger's Arch., 33; Mayer and Neuberg, Zeitschr. f. physiol. Chem., 29.

The potassium salt of skatoxyl-sulphuric acid is crystalline; it dissolves in water, but with difficulty in alcohol. A watery solution becomes deep violet with ferric chloride, and red with concentrated nitric acid. The salt is decomposed by concentrated hydrochloric acid with the separation of a red precipitate. The nature of this red coloring-matter produced by the decomposition of skatoxyl-sulphuric acid is not well known; neither has the relationship existing between this and other red coloring-matters in the urine been decided. On distillation with zinc-dust the skatol-chromogen yields skatol.

Urines containing skatoxyl are colored dark red to violet by JAFFÉ's indican test even on the addition of hydrochloric acid alone; with nitric acid they are colored cherry-red, and red on warming with ferric chloride and hydrochloric acid. The coloring-matter which yields skatol with zinc-dust may be removed from the urine by ether. Urines rich in skatoxyl darken from the surface downward when allowed to stand in the air, and may become reddish, violet, or nearly black. ROSIN is of the opinion that no skatol-chromogen exists in human urine, and that the observations made heretofore were due to a confusion with indigo red or urorosein. It cannot be disputed that derivatives of skatol occur in the urine, while the recent investigations of STAAL, GROSSER, PORCHER, and HERVIEUX[2] indicate that skatol-redand urorose in are identical or at least closely related pigments. Only the formation of skatol by distillation with zinc powder can be considered as a positive proof as to the skatol nature of a pigment.

SALKOWSKI[3] has demonstrated that the occurrence of *skatol-carboxylic acid* (indol acetic acid), $C_9H_8.N.COOH$, in normal urine is probable. This is also a product of putrefaction. When introduced into the animal body this acid reappears unchanged in the urine. With hydrochloric acid and very dilute ferric-chloride solution it gives an intense violet color to the solution. This test responds with a watery solution containing 1: 10 000 of skatol-carboxylic acid.

Aromatic Oxyacids. In the putrefaction of proteins in the intestine, *paraoxyphenyl-acetic acid*, $C_6H_4(OH).CH_2COOH$, and *paraoxyphenyl-propionic acid*, $C_6H_4(OH).C_2H_4.COOH$, are formed from tyrosine as an intermediate step, and these in great part pass unchanged into the urine. The quantity of these acids is usually very small. They are increased under the same conditions as the phenols, especially in acute phosphorus poisoning, in which the increase is considerable. A small portion of these oxyacids is combined with sulphuric acid.

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[1] Brieger, Ber. d. deutsch. chem. Gesellsch., **12**, and Zeitschr. f. physiol. Chem., **4**, 414; Mester, *ibid.*, **12**.

[2] Rosin, Virchow's Arch., **123**; Staal, Zeitschr. f. physiol. Chem., **46**; Grosser, *ibid.*, **44**; Porcher and Hervieux, Compt. rend., **138**, and Journ. de Physiol., **7**.

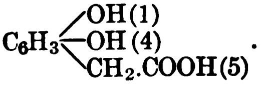
[3] Zeitschr. f. physiol. Chem., **9**.

Besides these two oxyacids which regularly occur in human urine we sometimes have other oxyacids in urines. To these belong *homogentisic acid* and *uroleucic acid*, the first of which forms the specific constituents of the urine in most cases of alcaptonuria, *oxymandelic acid*, found by SCHULTZEN and RIESS in urine in acute atrophy of the liver, *oxyhydroparacoumaric acid*, found by BLENDERMANN in the urine on feeding rabbits with tyrosine, *gallic acid*, which, according to BAUMANN,[1] sometimes appears in horse's urine, and *kynurenic acid* (oxyquinolincarboxylic acid), which up to the present time has been found only in dog's urine. Although all these acids do not belong to the physiological constituents of the urine, still they will be treated in connection with these.

Paraoxyphenylacetic Acid, $C_8H_8O_3=C_6H_4<\begin{matrix}OH\\CH_2.COOH\end{matrix}$, and **p-Oxyphenylpropionic Acid** (Hydroparacoumaric Acid), $C_9H_{10}O_3=C_6H_4<\begin{matrix}OH\\CH_2.CH_2COOH\end{matrix}$, are crystalline and are soluble both in water and in ether. The one melts at 148° C. and the other at 125° C. Both give a beautiful red coloration on being warmed with MILLON'S reagent.

To detect the presence of these oxyacids proceed in the following way (BAUMANN): Warm the urine for a while on the water-bath with hydrochloric acid in order to drive off the volatile phenols. After cooling shake three times with ether, and then shake the ethereal extracts with dilute soda solution, which dissolves the oxyacids, while the residue of the phenols which are soluble in ether remains. The alkaline solution of the oxyacids is now faintly acidified with sulphuric acid, shaken again with ether, the ether removed and allowed to evaporated the residue dissolved in a little water, and the solution tested with MILLON'S, reagent. The two oxyacids are best differentiated by their different melting-points. The reader is referred to other works for the method of isolating and separating these two oxyacids.

Homogentisic Acid (Dioxyphenylacetic Acid), $C_8H_8O_4=$



$C_6H_3\left\langle\begin{matrix}OH(1)\\OH(4)\\CH_2.COOH(5)\end{matrix}\right.$

. This acid, which was discovered by MARSHALL[2] and by him called *glycosuric acid*, was isolated in larger quantities by WOLKOW and BAUMANN in a case of alcaptonuria and carefully studied by them. They called it homogentisic acid because it is a homologue of gentisic acid, and they showed that the peculiar properties of so-called alcaptonuric urine in this case were due to this acid. This acid has later been found in many cases of alcaptonuria by EMBDEN, GARNIER and VOIRIN, OGDEN, GARROD, and many others. *Glycosuric acid*, isolated from alcaptonuric urine by GEYGER,[1] seems to be identical with homogentisic acid.

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[1] Schultzen and Riess, Chem. Centralbl., 1869; Blendermann, Zeitschr. f. physiol. Chem., 6, 267; Baumann, *ibid.*, 6, 193.

[2] The Medical News, Philadelphia, January 8, 1887.

The quantity of acid eliminated is increased by food rich in protein. On the ingestion of tyrosine by persons with alcaptonuria, WOLKOW and BAUMANN and EMBDEN observed a greater quantity of homogentisic acid in the urine. Since LANGSTEIN and E. MEYER showed in a case of alcaptonuria that the quantity of tyrosine in the protein, even when calculated to a maximum, was not sufficient to account for the quantity of homogentisic acid, and that therefore we must admit of another source (the phenylalanine) for the alcapton, FALTA and LANGSTEIN[2] have given a direct proof that homogentisic acid can also be formed from phenylalanine. Tyrosine and phenylalanine are quantitatively converted into homogentisic acid in alcaptonuria (FALTA). Dibromtyrosine, on the contrary, yields as little homogentisic acid as bromine or iodine derivatives of protein bodies (FALTA). According to the investigations of LANGSTEIN and MEYER, and especially of FALTA, different proteins yield varying quantities of homogentisic acid in alcaptonuria, and accordingly larger amounts in proportion as the protein is rich in tyrosine and phenylalanine.

WOLKOW and BAUMANN explain the formation of homogentisic acid from tyrosine by an abnormal fermentation in the upper parts of the intestine, but this view has now been generally rejected. Homogentisic acid is burnt in the healthy organism, and in consonance with the views of FALTA and LANGSTEIN alcaptonuria is considered as an anomaly in the metabolism. O. NEUBAUER and FALTA[3] found in experiments with different aromatic substances that the aromatic α-oxyacids as well as the α-amino acids derived from the protein bodies, are converted into homogentisic acid in the organism of alcaptonurics. It can be admitted with FALTA that the phenylalanine in the body by deamidation is converted into phenyl-α-lactic acid, $C_6H_5.CH_2.CHOH.COOH$, from which by taking up two hydroxyl groups dioxyphenyl-α-lactic acid (uroleucic acid), $(OH)_2C_6H_3.CH_2.CHOH.COOH$, is formed, and then from this by oxidation dioxyphenylacetic acid (homogentisic acid), $(OH)_2C_6H_3.CH_2.COOH$, is produced. Tyrosine also is supposed to undergo an analogous transformation whereby a removal of OH groups in the para position must be admitted and in both cases the homogentisic acid formed is under normal conditions further destroyed by a rupture of the benzene ring. The demolition of the tyrosine and the phenylalanine according to this view takes place in normal organisms by way of the alcaptonic acids, and the metabolic anomaly in alcaptonuria consists in that the demolition stops at this point and the property of the organism in alcaptonuria of rupturing the benzene ring is absent.

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[1] Wolkow and Baumann, Zeitschr. f. physiol. Chem., **15**; Embden, *ibid.*, **17** and **18**; Garnier and Voirin, Arch. de Physiol. (5), **4**; Ogden, Zeitschr. f. physiol. Chem., **20**; Geyger, cited from Embden, l c., **18**.

[2] Langstein and Meyer, Deutsch. Arch. f. klin. Med., **78**; Falta and Langstein, Zeitschr. f. physiol. Chem., **37**; Falta, Der Eiweiss-Stoffwechsel bei der Alkaptonurie, Habilitationsschrift, Naumburg a. S., 1904.

[3] Zeitschr. f. physiol. Chem., **42**.

GARROD,[1] who has observed several cases of alcaptonuria, has also tabulated about forty cases of alcaptonuria which he finds in the literature. From this he shows that the anomaly of the protein metabolism occurs oftener in males than in females, and also that blood relationship of the parents (first cousins) predisposes to alcaptonuria.

On fusing homogentisic acid with alkali it yields gentisic acid (hydroquinone-carboxylic acid) and hydroquinone. When introduced into the intestine of the dog a part is converted into toluhydroquinone, which is eliminated in the form of an ethereal sulphuric acid. Homogentisic acid has also been prepared synthetically by BAUMANN and FRÄNKEL,[2] starting with gentisic aldehyde.

Homogentisic acid crystallizes with 1 mol. of water in large, transparent prismatic crystals, which become non-transparent at the temperature of the room with the loss of water of crystallization. They melt at 146.5–147° C. They are soluble in water, alcohol, and ether, but nearly insoluble in chloroform and benzene. Homogentisic acid is optically inactive and non-fermentable. Its watery solution has the properties of so-called alcaptonuric urine. It becomes greenish brown from the surface downward on the addition of very little caustic soda or ammonia with excess of oxygen, and on shaking it quickly becomes dark brown or black. It reduces alkaline copper solutions with even slight heat, and ammoniacal silver solutions immediately in the cold. It does not reduce alkaline bismuth solutions. It gives a lemon-colored precipitate with MILLON's reagent, which becomes light brick-red on warming. Ferric chloride gives to the solution a blue color which soon disappears. On boiling with concentrated ferric-chloride solution an odor of quinone develops. With benzoyl chloride and caustic soda in the presence of ammonia we obtain the amide of dibenzoylhomogentisic acid, which melts at 204° C., and which can be used in the isolation of the acid from the urine, and also for its detection (ORTON and GARROD). Among the salts of this acid must be mentioned the lead salt containing water of crystallization and 34.79 per cent Pb. This salt melts at 214–215° C.

In order to prepare the acid, heat the urine to boiling, add 5 grams of lead acetate for every 100 c.c., filter as soon as the lead acetate has dissolved, and allow the filtrate to stand in a cool place for twenty-four hours until it crystallizes (GARROD). The dried, powdered lead salt is suspended in ether and decomposed by H_2S. After the spontaneous evaporation of the ether the acid is obtained in nearly colorless crystals (ORTON and GARROD [1]).

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[1] Med. chirurg. Transact., 1899 (where all known cases are tabulated); also The Lancet, 1901 and 1902; Garrod and Hele, Journ. of Physiol., 33.

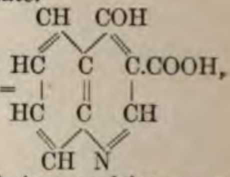
[2] Zeitschr. f. physiol. Chem., 20.

In regard to the quantitative estimation we proceed according to the suggestion of BAUMANN by titrating the acid with a N/10 silver solution. As regards details of this method the reader is referred to the works of BAUMANN, C. TH. MÖRNER and MITTELBACH, GARROD and HURTLEY. DENIGÈS [2] has suggested another method.

Uroleucic acid, $C_9H_{10}O_5$, is, according to HUPPERT, probably a dioxyphenyllactic acid, $C_6H_3(OH)_2.CH_2.CH(OH).COOH$. This acid was first prepared by KIRK from the urine of children with alcaptonuria, which also contained homogentisic acid. LANGSTEIN and MEYER [3] have also found a small amount of this acid in a case of alcaptonuria studied by them. It melts at 130–133° C. Otherwise, in regard to its behavior with alkalies, with access of air, and also with alkaline copper solutions and ammoniacal silver solutions, and also MILLON'S reagent, it is similar to homogentisic acid.

Oxymadelic acid, $C_8H_8O_4$, paraoxyphenylglycolic acid, $HO.C_6H_4.CH(OH)COOH$, is, as above stated, found in the urine in acute atrophy of the liver. The acid crystallizes in silky needles. It melts at 162° C., dissolves readily in hot water, less in cold water, and readily in alcohol and ether, but not in hot benzene. It is precipitated by basic lead acetate, but not by lead acetate.

Kynurenic acid (γ-oxy-β-quinolincarboxylic acid), $C_{10}H_7NO_3$ =



has only been found thus far in dogs' urine; its quantity is increased by meat feeding. According to the observations of GLAESSNER and LANGSTEIN, the mother-substance seems to be contained among the products of pancreatic digestion which are soluble in alcohol and precipitable by acetone. ELLINGER [4] has recently been able to show positively that tryptophane is the mother-substance of this acid. By the introduction of tryptophane in the organism he has shown the formation of a kynurenic acid not only in dogs but also in rabbits. The acid is crystalline, does not dissolve in cold water, rather well in hot alcohol, and yields a barium salt which crystallizes in triangular, colorless plates. On heating it melts and decomposes into CO_2 and kynurin. On evaporation to dryness on the water-bath with hydrochloric acid and potassium chlorate a reddish residue is obtained which becomes first brownish green and then emerald-green on adding ammonia (JAFFÉ'S reaction [5]).

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[1] Orton and Garrod, Journ. of Physiol., **27**; Garrod, *ibid.*, **23**.

[2] Mittelbach, Deutsch. Arch. f. klin. Med., **71** (which contains the work of Baumann and Mörner); Garrod and Hurtley, Journ. of Physiol., **33**; Denigès, Chem. Centralbl. 1897, **1**, 338.

[3] Huppert, Zeitschr. f. physiol. Chem., **23**; Kirk, Brit. Med. Journ., 1886 and 1888; Langstein and Meyer, l. c.

[4] Glaessner and Langstein, Hofmeister's Beiträge, **1**; Ellinger, Ber. d. d. chem. Gesellsch., **37**, 1804, and Zeitschr. f. physiol. Chem., **43**. The older literature on kynurenic acid may be found in Josephsohn, Beiträge zur Kenntnis der Kynurensäure ausscheidung beim Hunde, Inaug.-Dissert., Königsberg, 1898.

[5] Zeitschr. f. physiol. Chem., **7**. In regard to kynurenic acid, see also Huppert-Neubauer, 10. Aufl., and Mendel and Jackson, Amer. Journ. of Physiol., **2**; Mendel and Schneider, *ibid.*, **5**; Camps, Zeitschr. f. physiol. Chem., **33**.

Urinary Pigments and Chromogens. The yellow color of normal urine depends perhaps upon several pigments, but in greatest part upon *urochrome.* Besides this the urine seems to contain a very small quantity of *hæmatoporphyrin* as a regular constituent. *Uroerythrin* also is of frequent occurrence in normal urine, but not always. Finally, the excreted urine when exposed to the action of light regularly contains a yellow pigment, *urobilin,* which is derived from a chromogen, *urobilinogen,* by the action of light (SAILLET) and air (JAFFÉ, DISQUÉ,[1]) and others. Besides this chromogen, urine contains various other bodies from which coloring-matters may be produced by the action of chemical agents. Humin substances (perhaps in part from the carbohydrates of the urine) may be formed by the action of acids (v. UDRÁNSZKY) without regard to the fact that such substances may sometimes originate from the reagents used, as from impure amyl alcohol (v. UDRÁNSZKY [2]). To these humin bodies developed by the action of acid in normal urine when exposed to the air must be added the *urophain* of HELLER, the various *uromelanins* and other bodies described by different investigators (PLÓSZ, THUDICHUM, SCHUNK [3]). Indigo-blue (*uroglaucin* of HELLER, *urocyanin, cyanurin,* and other coloring-matters of older investigators [4]) is split off from the indoxyl-sulphuric acid or indoxyl-glucoronic acid. Red coloring-matter may be formed from the conjugated indoxyl and skatoxyl acids, and *urohodin* (HELLER), *urorubin* (PLOSZ), *urohæmatin* (HARLEY), and perhaps also *urorosein* (NENCKI and SIEBER [5]) probably have such an origin.

We cannot discuss more in detail the different coloring-matters obtained as decomposition products from normal urine. Hæmatoporphyrin has already been referred to in a previous chapter (VI) and will best be described in connection with the pathological pigments. It only remains to describe urochrome, urobilin, and uroerythrin.

Urochrome is the name given by GARROD to the yellow pigment of the urine. THUDICHUM [6] had previously given the same name to a less pure pigment isolated by himself. According to GARROD urochrome is free from iron, but contains nitrogen. It stands, it seems, in close relationship to urobilin, as GARROD has obtained a urobilin-like pigment by the action of impure aldehyde on urochrome, and RIVA [7] claims that urobilin yields a body similar to urochrome on careful oxidation with permanganate. According to GARROD urobilin can be converted into urochrome by evaporating its aqueous solution containing some ether on the water-bath. The fact that urochrome can be transformed into urobilin by means of active acetaldehyde may be used, according to GARROD, as a means of detecting urochrome.

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[1] Jaffé, Centralbl. f. d. med. Wissensch. 1868 and 1869, and Virchow's Arch., **47**; Disqué, Zeitschr. f. physiol. Chem., **2**; Saillet, Revue de médecine, **17**, 1897.

[2] v. Udránszky, Zeitschr. f. physiol. Chem., **11**, **12**, and **13**.

[3] Plósz, Zeitschr. f. physiol. Chem., 8; Thudichum, Brit. Med. Journ., **201**, and Journ. f. prakt. Chem., **104**; Schunk, cited from Huppert-Neubauer, 10. Aufl., 509.

[4] See Huppert-Neubauer, 161.

[5] In regard to this and other red pigments, see Huppert-Neubauer, 593 and 597; Nencki and Sieber, Journ. f. prakt. Chem. (2), **26**.

[6] Garrod, Proceed. Roy. Soc., **55**; Thudichum, l. c.

[7] Garrod, Journ. of Physiol., **21** and **29**; Riva, cited from Huppert-Neubauer, **524**.

Urochrome is, according to GARROD, amorphous, brown, very readily soluble in water and ordinary alcohol, but less soluble in absolute alcohol. It dissolves but slightly in acetic ether, amyl alcohol, and acetone, while it is insoluble in ether, chloroform, and benzene. Urochrome is precipitated by lead acetate, silver nitrate, mercuric acetate, phosphotungstic and phosphomolybdic acids. On saturating the urine with ammonium sulphate a great part of the urochrome remains in solution. It does not show any absorption-bands and does not fluoresce after the addition of ammonia and zinc chloride. Urochrome is very readliy decomposed, with the formation of brown substances, by the action of acids. According to KLEMPERER,[1] urochrome contains 4.2 per cent nitrogen.

Urochrome can be prepared according to a rather complicated method which is based upon the fact that the substance remains in great part in solution on saturating the urine with ammonium sulphate. If the proper quantity of alcohol is added to the filtrate, a clear, yellow alcoholic layer forms on the salt solution, which contains the urochrome and which can be used for the further preparation of the latter (see GARROD, l. c.). KLEMPERER, on the contrary, removes the pigment from the urine by means of animal charcoal, washes it with water to remove the indican and other bodies, and then extracts with alcohol and uses this alcoholic extract for the further purification according to GARROD.

The urochrome can be quantitatively estimated, according to KLEMPERER, by a colorimetric method, using a solution of true yellow G. If 0.1 gram of this dye is dissolved in 1 liter of water and 5 c.c. of this solution diluted to 50 c.c. with water, then this solution has the same color and shade as a 0.1 per cent urochrome solution. The urine must be diluted with water until it has the same depth of color. The comparison is performed in vessels with parallel walls.

Urobilin is the pigment first isolated from the urine by JAFFÉ,[2] and which is characterized by its strong fluorescence and by its absorption-spectrum. Various investigators have prepared from the urine by different methods pigments which differed slightly from each other but behaved essentially like JAFFÉ's urobilin. Thus different urobilins have been suggested, such as normal, febrile, physiological, and pathological urobilins.[3]

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[1] Berlin. klin. Wochenschr., **40**.

[2] Centralbl. f. d. med. Wissensch., 1868 and 1869, and Virchow's Arch., **47**.

[3] See MacMunn, Proc. Roy. Soc., **31** and **35**; Ber. d. deutsch. chem. Gesellsch., **14**, and Journ. of Physiol., **6** and **10**; Bogomoloff, Maly's Jahresber., **22**; Eichholz, Journ. of Physiol., **14**; Ad. Jolles, Pflüger's Arch., **61**.

The possibility of the occurrence of different urobilins in the urine cannot be denied; but as urobilin is a readily changeable body and difficult to purify from other urinary pigments, the question as to the occurrence of different urobilins must still be considered open. According to SAILLET[1] no urobilin exists originally in human urine, but only the mother-substance of the same, urobilinogen, from which the urobilin is formed in the excreted urine by the influence of light.

Urobilin-like bodies, so-called *urobilinoids*, have been prepared from bile-pigments as well as blood-pigments, and indeed by oxidation as well as reduction. MALY obtained his hydrobilirubin by the reduction of bilirubin with sodium amalgam, and DISQUÉ obtained a product which is still more similar to urobilin, while STOKVIS prepared by the oxidation of cholecyanin with a little lead peroxide a choletelin which acted very much like urobilin. HOPPE-SEYLER, LE NOBEL, NENCKI and SIEBER have obtained urobilinoid bodies by the reduction of hæmatin and hæmatoporphyrin with tin or zinc and hydrochloric acid, while MACMUNN[2] obtained by the oxidation of hæmatin with hydrogen peroxide in alcohol containing sulphuric acid a pigment which seemed to be identical with urinary urobilin. It is apparent that all these urobilins cannot be identical.

Many investigators declare that urobilin is identical with hydrobilirubin, but according to the researches of HOPKINS and GARROD[3] this view is not correct, because, irrespective of other small differences, each body has an essentially distinct composition. Hydrobilirubin contains C 64.68, H 6.93, N 9.22 (MALY), while urinary urobilin, on the contrary, contains C 63.46, H 7.67, N 4.09 per cent. The urobilin from fæces, *stercobilin*, has the same composition as urinary urobilin with 4.17 per cent nitrogen.

Urinary urobilin may not be identical with hydrobilirubin, but this does not exclude the possibility that urobilin, according to the generally admitted view, is derived from bilirubin (although not by simple reduction and taking up water) in the intestine. Several physiological as well as clinical observations[4] speak for this view, among which we must mention the regular appearance in the intestinal tract of stercobilin, undoubtedly derived from the bile-pigments and having the same composition as urinary urobilin, the absence of urobilin in the urine of new-born infants as well as on the complete exclusion of bile from the intestine, and also the increased elimination of urobilin with strong intestinal putrefaction. On the other hand there are investigators who, basing their opinion on clinical observations, deny the intestinal origin of urobilin and claim that the urobilin is derived from a transformation of the bilirubin elsewhere than in the intestine, by an oxidation of the bile-pigment or by a transformation of the blood-pigments.[1] The possibility of a different mode of formation of urinary urobilin in disease is not to be denied; but there is no doubt that this pigment is formed from the bile-pigments in the intestine under physiological conditions.

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[1] Revue de médecine, 17, 1897.

[2] Maly, Ann. d. Chem. u. Pharm., 163; Disqué, Zeitschr. f. physiol. Chem., 2; Stokvis, Centralbl. f. d. med. Wissensch., 1873, 211 and 449; Hoppe-Seyler, Ber. d. deutsch. chem. Gesellsch., 7; Le Nobel, Pflüger's Arch., 40; Nencki and Sieber, Monatshefte f. Chem., 9, and Arch. f. exp. Path. u. Pharm., 24; MacMunn, Proc. Roy. Soc., 31.

[3] Journ. of Physiol., 22.

[4] See Fr. Müller, Schles. Gesellsch. f. vaterl. Kultur, 1892; D. Gerhardt, "Ueber Hydrobilirubin und seine Bezieh. zum Ikterus" (Inaug.-Diss., Berlin, 1889); Beck, Wien. klin. Wochenschr., 1895; Harley, Brit. Med. Journ., 1896.

The quantity of urobilin in the urine under physiological conditions is very variable. SAILLET found 30–130 milligrams and G. HOPPE-SEYLER 80–140 milligrams in one day's urine.

There are numerous observations on the elimination of urobilin in disease, especially by JAFFÉ, DISQUÉ, GERHARDT, G. HOPPE-SEYLER,[2] and others. The quantity is increased in hemorrhage and in such diseases where the blood-corpuscles are destroyed, as is the case after the action of certain bood-poisons, such as antifibrine and antipyrine. It is also increased in fevers, cardiac diseases, lead colic, atrophic cirrhosis of the liver, and is especially abundant in so-called urobilin icterus.

The properties of urobilin may be different, depending upon the method of preparation and the character of the urine used; therefore only the most important properties will be given. Urobilin is amorphous, brown, reddish brown, red, or reddish yellow, depending upon the method of preparation. It dissolves readily in alcohol, amyl alcohol, and chloroform, but less readily in ether or acetic ether. It is less soluble in water, but the solubility is augmented by the presence of neutral salts. It may be completely precipitated from the urine by saturating with ammonium sulphate, especially after the addition of sulphuric acid (MÉHU[3]). It is soluble in alkalies, and is precipitated from the alkaline solution by the addition of acid. It is partly dissolved by chloroform from an acid (watery-alcoholic) solution; alkali solutions remove the urobilin from the chloroform. The neutral or faintly alkaline solutions are precipitated by certain metallic salts (zinc and lead), but not by others, such as mercuric sulphate. Urobilin is precipitated from the urine by phosphotungstic acid. It does not give GMELIN's test for bile-pigments. It gives, on the contrary, a reaction which may be mistaken for the biuret test, by the action of copper sulphate and alkali.[1]

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[1] In regard to the various theories as to the formation of urobilin, see Harley, Brit. Med. Journ., 1896; A. Katz., Wien. med. Wochenschr., 1891, Nos. 28–32; Grimm, Virchow's Arch., **132**; Zoja, Conferenze cliniche italiane, Ser. 1a, **1**.

[2] In regard to the literature on this subject we refer the reader to D. Gerhardt, "Ueber Hydrobilirubin und seine Beziehungen zum Ikterus" (Berlin, 1889), and also G. Hoppe-Seyler, Virchow's Arch., **124**.

[3] Journ. de Pharm. et Chim., 1878, cited from Maly's Jahresber., 8.

Neutral alcoholic urobilin solutions are in strong concentration brownish yellow, in great dilution yellow or rose-colored. They have a strong green fluorescence. The acid alcoholic solutions are brown, reddish yellow, or rose-red, according to concentration. They are not fluorescent, but show a faint absorption-band, γ, between *b* and *F*, which borders on *F*, or in greater concentration extends over *F*. The alkaline solutions are brownish yellow, yellow, or (the ammoniacal) yellowish green, according to concentration. If some zinc-chloride solution is added to an ammoniacal solution of the pigment it becomes red and shows a beautiful green fluorescence. This solution, as also that made alkaline with fixed alkalies, shows a darker and more sharply defined band, δ, between *b* and *F*, almost midway between *E* and *F*. If a sufficiently concentrated solution of urobilin alkali is carefully acidified with sulphuric acid it becomes cloudy and shows a second band exactly at *E* and connected with γ by a shadow (Garrod and Hopkins, Saillet[2]).

Urobilinogen is colorless or is only slightly colored. Like urobilin it is precipitated from the urine by saturating with ammonium sulphate. According to Saillet it may be extracted by acetic ether from urine acidified with acetic acid. It dissolves also in chloroform, ethyl ether, and amyl-alcohol. It shows no absorption-bands and is readily converted into urobilin by the influence of sunlight and oxygen, and, according to Neubauer and Bauer,[3] gives the Ehrlich reaction with dimethylamidobenzaldehyde and hydrochloric acid (see below).

In preparing urobilin from normal urine, precipitate the urine with basic lead acetate (Jaffé), wash the precipitate with water, dry at the ordinary temperature, then boil it with alcohol, and decompose it when cold with alcohol containing sulphuric acid. The filtered alcoholic solution is diluted with water, saturated with ammonia, and then treated with zinc-chloride solution. This new precipitate is washed free from chlorine with water, boiled with alcohol, dried, dissolved in ammonia, and this solution precipitated with sugar of lead. This precipitate, which is washed with water and boiled with alcohol, is decomposed by alcohol containing sulphuric acid, the filtered alcoholic solution is mixed with ½ vol. chloroform, diluted with water, and shaken repeatedly, but not too energetically. The urobilin is taken up by the chloroform. This last is washed once or twice with a little water and then distilled, leaving the urobilin. The pigment may be precipitated directly from the urine rich in urobilin by ammonia and zinc chloride, and the precipitate treated as above described (Jaffé).

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[1] See Salkowski, Berlin. klin. Wochenschr., 1897, and Stokvis, Zeitschr. f. Biologie, 34.

[2] Garrod and Hopkins, Journ. of Physiol., 20; Saillet, l. c.

[3] Neubauer, cited from Centralbl. f. Physiol., 19, 145; Bauer, cited from Biochem. Centralbl., 4, 390.

The method suggested by **Méhu** (precipitation with ammonium sulphate) has been modified by **Garrod** and **Hopkins** in that they first remove the uric acid by saturating with ammonium chloride and then saturating the filtrate with ammonium sulphate. The precipitated urobilin is thus made purer than by saturating with the sulphate directly. The urobilin is extracted from the dried precipitate by a great deal of water, reprecipitated by ammonium sulphate, and this procedure repeated several times if necessary. The dried precipitate finally obtained is dissolved in absolute alcohol. In regard to small details, and to a second method suggested by these experimenters, we refer to the original work.[1]

Saillet extracts the urobilinogen from the urine by shaking with acetic ether, using a kerosene-oil light.[2]

The color of the acid or alkaline solution, the beautiful fluorescence of the ammoniacal solution treated with zinc chloride, and the absorption-bands of the spectrum, all serve as means of detecting urobilin. In fever-urines the urobilin may be detected directly or after the addition of ammonia and zinc chloride by its spectrum. It may also sometimes be detected in normal urine, either directly or after the urine has stood exposed to the air until the chromogen has been converted into urobilin. If it cannot be detected by means of the spectroscope, then the urine may be treated with a mineral acid and shaken with ether or, still better, with amyl alcohol. The amyl-alcohol solution is, either directly or after addition of a strongly ammoniacal alcoholic solution of zinc chloride, tested spectroscopically. According to **Schlesinger**[3] it can be readily detected if the urine is precipitated by an equal volume of a 10 per cent solution of zinc acetate in absolute alcohol. Disturbing bodies are here precipitated and the filtrate gives the fluorescence directly, and also the spectrum. **Grimbert**[4] has given another comparatively simple method.

In the quantitative estimation of urobilin we proceed as follows, according to G. **Hoppe-Seyler**:[5] 100 c.c. of the urine is acidified with sulphuric acid and saturated with ammonium sulphate. The precipitate is collected on a filter after some time, washed with a saturated solution of ammonium sulphate, and repeatedly extracted with equal parts of alcohol and chloroform after pressing. The filtered solution is treated with water in a separatory funnel until the chloroform separates well and becomes clear. The chloroform solution is evaporated on the water-bath in a weighed beaker, the residue dried at 100° C., and then extracted with ether. The ethereal extract is filtered, the residue on the filter dissolved in alcohol, and transferred to the beaker and evaporated, then dried and weighed. According to this method G. **Hoppe-Seyler** found 0.08–0.14 gram of urobilin in one day's urine of a healthy person, or an average of 0.123 gram.

Urobilin may also be determined spectrophotometrically according to **Fr. Müller** or **Saillet**.[6] **Saillet** found that the limit for the perceptibility of the absorption-bands of an acid-urobilin solution lies in a concentration of 1 milligram of urobilin in 22 c.c. of solution when the thickness of the layer of fluid is 15 mm. In a quantitative estimation the urobilin solution is diluted to this limit and then the quantity of urobilin calculated from the extent of dilution. The freshly voided urine, shielded from light, is acidified with acetic acid, completely extracted in kerosene-oil light with acetic ether, and the dissolved urobilinogen oxidized to urobilin with nitric acid. On the addition of ammonia and shaking with water the urobilin passes into the watery solution. This is acidified with hydrochloric acid and diluted until the above limit is reached.

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[1] Journ. of Physiol., **20**.

[2] In regard to this and other methods, we must refer the reader to special works.

[3] Deutsch. med. Wochenschr., 1903.

[4] See Chem. Centralbl., 1904, **1**, 1623.

[5] Virchow's Arch., **124**.

[6] Fr. Müller, see Huppert-Neubauer, 861; Saillet, l. c.

Uroerythrin is the pigment which often gives the beautiful red color to the urinary sediments (*sedimentum lateritium*). It also frequently occurs, although only in very small quantities, dissolved in normal urines. The quantity is increased after great muscular activity, after profuse perspiration, immoderate eating, or partaking of alcoholic drinks, as well as after digestive disturbances, fevers, circulatory disturbances of the liver, and in many other pathological conditions.

Uroerythrin, which has been especially studied by ZOJA, RIVA, and GARROD,[1] has a pink color, is amorphous, and is very quickly destroyed by light, especially when in solution. The best solvent is amyl alcohol; acetic ether is not so good, and alcohol, chloroform, and water are even less valuable. The very dilute solutions show a pink color; but on greater concentration they become reddish orange or bright red. They do not fluoresce either directly or after the addition of an ammoniacal solution of zinc chloride; but they have a strong absorption, beginning in the middle between *D* and *E* and extending to about *F*, and consisting of two bands which are connected by a shadow between *E* and *b*. Concentrated sulphuric acid colors a uroerythrin solution a beautiful carmine-red; hydrochloric acid gives a pink color. Alkalies make its solutions grass-green, and often a play of colors from pink to purple and blue is observed. PORCHER and HERVIEUX[2] claim that uroerythrin is a skatol pigment.

In preparing uroerythrin according to GARROD, the sediment is dissolved in water at a gentle heat and saturated with ammonium chloride, which precipitates the pigment with the ammonium urate. This is purified by repeated solution in water and precipitation with ammonium chloride until all the urobilin is removed. The precipitate is finally extracted on the filter in the dark with warm water, filtered, then diluted with water, any hæmatoporphyrin remaining being removed by shaking with chloroform; the precipitate is then faintly acidified with acetic acid and shaken with chloroform, which takes up the uroerythrin. The chloroform is evaporated in the dark at a gentle heat.

Volatile fatty acids, such as formic acid, acetic acid, and perhaps also butyric acid, occur under normal conditions in human urine (v. JAKSCH), also in that of dogs and herbivora (Schotten). The acids poorest in carbon, such as formic acid and acetic acid, are more constant in the body than those richer in carbon, and therefore the relatively greater part of these pass unchanged into the urine (Schotten). Normal human urine contains besides these bodies others which yield acetic acid when oxidized by potassium dichromate and sulphuric acid (v. Jaksch). The quantity of volatile fatty acids in normal urine calculated as acetic acid is, according to v. Jaksch, 0.008–0.009 gram per twenty-four hours; according to v. Rokitansky, 0.054 gram; and according to Magnus-Levy, 0.060 gram. The quantity is increased by exclusively farinaceous food (Rokitansky), in fever and in certain diseases, while in others it is diminished (v. Jaksch, Rosenfeld). Large amounts of volatile fatty acids are produced in the alkaline fermentation of the urine, and the quantity is 6–15 times as large as in normal urine (Salkowski [1]). *Non-volatile fatty acids* have been detected as normal constituents of urine by K. Mörner and Hybbinette.[2]

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[1] Zoja, Arch. ital. di clinica med., 1893, and Centralbl. f. d. med. Wissensch., 1892; Riva, Gaz. med. di Torino, Anno 43, cited from Maly's Jahresber., 24; Garrod, Journ. of Physiol., 17 and 21.

[2] Journ. de Physiol., 7.

Paralactic Acid. It is claimed that this acid occurs in the urine of healthy persons after very fatiguing marches (Colasanti and Moscatelli). It is found in larger amounts in the urine in acute phosphorus-poisoning or acute yellow atrophy of the liver (Schultzen and Riess), and especially abundant in eclampsia (Zweifel). According to the investigations of Hoppe-Seyler, Araki, and v. Terray lactic acid passes into the urine as soon as the supply of oxygen is decreased in any way, and this probably explains the occurrence of lactic acid in the urine after epileptic attacks (Inouye and Saiki). Minkowski [3] has shown that lactic acid occurs in the urine in large quantities on the extirpation of the liver of birds.

Glycerophosphoric acid occurs as traces in the urine,[4] and it is probably a decomposition product of lecithin. The occurrence of *succinic acid* in normal urine is a subject of discussion.

Carbohydrates and Reducing Substances in the Urine. The occurrence of *dextrose* as traces in normal urine is highly probable, as the investigations of Brücke, Abeles, and v. Udránszky show. The last investigator has also shown the habitual occurrence of carbohydrates in the urine, and their presence has been positively proved by the investigations of Baumann and Wedenski, and especially by Baisch. Besides dextrose normal urine contains, according to Baisch, another not well-studied variety of sugar; according to Lemaire, probably isomaltose is present, and besides this a dextrin-like carbohydrate (animal gum), as shown by Landwehr, Wedenski, and Baisch. The quantity of carbohydrates eliminated under normal conditions in the twenty-four hours' urine and determined by the benzoylation method, which is perhaps not sufficiently trustworthy, varies considerably between 1.5 and 5.09 grams.[1]

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[1] v. Jaksch, Zeitschr. f. physiol. Chem., **10**; Schotten, *ibid.*, **7**; Rokitansky, Wien. med. Jahrbuch, 1887; Salkowski, Zeitschr. f. physiol. Chem., **13**; Magnus-Levy, Salkowski's Festschrift, 1904; Rosenfeld, Deutsch. med. Wochenschr., **29**.

[2] Skand. Arch. f. Physiol., **7**.

[3] Colasanti and Moscatelli, Moleschott's Untersuch., **14**; Schultzen and Reiss, Chem. Centralbl., 1869; Zweifel, Arch. f. Gynäkol, **76**; Araki, Zeitschr. f. physiol. Chem., **15**, **16**, **17**. **19**. See also Irisawa, *ibid.*, **17**; v. Terray, Pflüger's Arch., **65**; Schütz, Zeitschr. f. physiol. Chem., **19**; Inouye and Saiki, *ibid.*, **37**; Minkowski, Arch. f. exp. Path. u. Pharm., **21** and **31**.

[4] See Pasqualis, Maly's Jahresber., **24**.

The precipitate obtained from concentrated urine by the aid of alcohol and whose nitrogen (colloidal nitrogen according to SALKOWSKI) in normal urine amounts to 2.34–4.08 per cent of the total nitrogen and in pathological urines to 8–9 per cent, and in a case of acute yellow atrophy of the liver to 21.8 per cent, contains, according to SALKOWSKI,[2] a nitrogenous carbohydrate which has strong reducing action upon alkaline copper solutions after cleavage with hydrochloric acid.

Besides traces of sugar and the reducing substances previously mentioned, uric acid and creatinine, the urine contains still other bodies of this character. These latter are partly conjugated compounds of *glucuronic acid*, $C_6H_{10}O_7$, which is closely allied to dextrose. The reducing power of normal urine corresponds, according to various investigators, to 1.5–5.96 p. m. dextrose.[3] That portion of the reduction belonging to dextrose alone is equal to 0.1–0.6 p. m.

Several new methods for the determination of the reducing power of the urine have been suggested.[4]

Conjugated glucoronates occur, as indicated by FLUCKIGER and first positively shown by MAYER and NEUBERG,[5] in very small amounts in normal urine. They occur chiefly as phenol- and only very small amounts of indoxyl- or skatoxylglucuronates. The quantity of glucuronic acid obtained from the conjugated glucuronates is estimated as 0.04 p. m. by MAYER and NEUBERG. Besides these conjugated glucuronates perhaps sometimes the urine contains the urea glucuronic acid, the ureidoglucuronic acid prepared synthetically by NEUBERG and NEIMANN.[6]

Very large amounts of these conjugated glucuronates occur in the urine, on the other hand, after partaking of various therapeutic agents and other substances, such as chloral hydrate, camphor, naphthol, borneol, turpentine, morphine, and many other substances. The elimination of glucuronic acid may be markedly increased in severe disturbances of the respiration, severe dyspnœa, in diabetes mellitus, and by the direct introduction of large amounts of dextrose. According to P. MAYER, as stated on page 122, in the oxidation of dextrose a part of it forms glucuronic acid, hence it is to be expected that the glucuronic acid can in part be derived from the dextrose. As a conjugation of the glucuronic acid with other bodies, such as aromatic atomic complexes, prevents the combustion of this acid in the animal body, it ought to follow that after the introduction of such an atomic complex in the body during a glycosuria a corresponding reduction of the glucose elimination would take place with the increased excretion of conjugated glucuronates. In order to prove this possibility O. LOEWI[1] fed dogs with camphor during phlorhizin diabetes and found that the above expectation was not realized. Although large quantities of campho-glucuronic acid were excreted, the sugar excretion was only slightly diminished and not in proportion to the quantity of conjugated glucuronate excreted. These negative results are contradicted by the positive results obtained by PAUL MAYER.[2] Normally rabbits convert nearly all the camphor introduced into conjugated glucuronic acid. According to MAYER if we allow a rabbit to starve several days the animal becomes so poor in the mother-substance (glycogen) yielding the glucuronic acid that the introduction of camphor only brings about an elimination of small quantities of glucuronic acid. By the simultaneous administration of camphor and dextrose while starvation is going on, the elimination of glucuronic acid rises again to the same height as it was before the starvation period. This shows that the sugar had conjugated itself with the camphor as glucuronic acid. HILDEBRANDT[3] has also made experiments showing that glucuronic acid can very likely be formed from sugar. The observations of MAYER are not substantiated by the recent investigations of FENYVESSY,[4] and the statements on this question are contradictory.

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[1] Lemaire, Zeitschr. f. physiol. Chem., **21**; Baisch, *ibid.*, **18**, **19**, and **20**. In these as well as in Treupel, *ibid.*, **16**, the works of other investigators are cited. See also v. Alfthan, Deutsch. med. Wochenschr., **26**.

[2] Berlin. klin. Wochenschr., 1905.

[3] Flückiger, Zeitschr. f. physiol. Chem., **9**. See also Huppert-Neubauer, page 72.

[4] See Rosin, Münch. med. Wochenschr., **46**; Niemilowicz, Zeitschr. f. physiol. Chem., **36**; Niemilowicz with Gittelmacher-Wilenko, *ibid.*, **36**, and Hélier, Compt. rend., **129**.

[5] Flückiger, l. c.; Mayer and Neuberg, Zeitschr. f. physiol. Chem., **29**.

[6] Zeitschr. f. physiol. Chem., **44**.

The conjugated glucuronic acids are formed, based upon the investigations of SUNDWIK, FISCHER and PILOTY,[5] by a combination tagkin place first between the conjugator and the dextrose by means of the aldehyde group, and then the end alcohol group, CH_2OH, is oxidized to COOH. The conjugated glucuronic acids at least in most cases seem to be constructed after the glucoside type, a view which has received further support by the synthesis of phenolglucuronic acid and euxanthonglucuronic acids by NEUBERG and NEIMANN.[6] Based upon their cleavage (as far as they have been investigated) by kephir lactase and emulsion, but not by yeast lactase (NEUBERG and WOHLGEMUTH[7]), the conjugated glucuronic acids must belong to the β-series of glucosides. The ureidoglucuronic acid is still not constructed upon the glucoside type, but according to the aldehydimine type, $H_2N.CO.N.CH.(CHOH)_4COOH$. The reducing urochloralic acid can hardly be built upon the glucoside type.

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[1] Arch. f. exp. Path. u. Pharm., **47**.

[2] Zeitschr. f. klin. Med., **47**.

[3] Arch. f. exp. Path. u. Pharm., **44**.

[4] See Maly's Jahresber., **34**.

[5] E. Sundwik, Akademische Abhandlung Helsingfors, 1886; see also Maly's Jahresber., **16**, 76; Fischer and Piloty, Ber. d. d. chem. Gesellsch., **24**.

[6] Zeitschr. f. physiol. Chem., **44**.

[7] See Neuberg, Ergebnisse der Physiologie, Bd. **3**, Abt. **1**, 444.

According to the body with which they are conjugated the glucuronates show different behavior; they all rotate the plane of polarization to the left, while the glucuronic acid itself is dextrorotatory. On taking up water they split into glucuronic acid and the conjugated group. They are precipitated by basic lead acetate or by basic lead acetate and ammonia. Most of the conjugated glucuronic acids do not have a reducing action. A few reduce copper oxide and certain other metallic oxides in alkaline solution and hence cause errors in the investigation of the urine for sugar. As the detection of conjugated glucuronic acids is connected with the tests for sugar in the urine, we will treat of this in connection with these tests.

Organic combinations containing sulphur of unknown kind, which may in small part consist of *sulphocyanides*, 0.04 (GSCHEIDLEN)–0.11 p. m. (I. MUNK [1]), *cystine* or bodies related to it, *taurine derivatives*, *chrondroitin-sulphuric acid* and *protein bodies*, but in greater part are made up of *antoxyproteic acid*, *oxyproteic acid*, *alloxyproteic acid*, and *uroferric acid*, are found in human as well as in animal urines. The sulphur of these mostly unknown combinations has been called "neutral," to differentiate it from the "acid" sulphur of the sulphate and ethereal-sulphuric acids (SALKOWSKI [2]). The neutral sulphur in normal urine as determined by SALKOWSKI is 15 per cent, by STADTHAGEN 13.3–14.5 per cent, and by LÉPINE 20 per cent, and HARNACK and KLEINE [3] 19–24 per cent of the total sulphur. In starvation, according to FR. MÜLLER, with insufficient supply of oxygen (REALE and BOERI, HARNACK and KLEINE), as in chloroform narcosis (KAST and MESTER), as also after the introduction of sulphur (PRESCH and YVON [4]), the quantity of neutral sulphur is increased. The quantity of neutral sulphur varies, according to BENEDICT, within rather narrow limits and especially, according to FOLIN, is dependent to a less degree than the sulphate excretion upon the extent of the protein metabolism. The relationship between the neutral and acid sulphur depends in the first place upon the extent of the sulphuric-acid excretion. According to HARNACK and KLEINE,[5] the relationship of the oxidized sulphur to the total sulphur changes always in the same way as the relationship of the nitrogen of the urea to the total nitrogen. The more unoxidized sulphur is eliminated the more abundant are the nitrogen compounds, not urea, in the urine—a statement which coincides with recent observations showing that the neutral sulphur originates chiefly from the oxyproteic acid, the alloxyproteic acid, and the uroferric acid.

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[1] Gscheidlen, Pflüger's Arch., **14**; Munk, Virchow's Arch., **69**.

[2] *Ibid.*, 58, and Zeitschr. f. physiol. Chem., **9**.

[3] Stadthagen, Virchow's Arch., **100**; Lépine, Compt. rend., **91** and **97**; Harnack and Kleine, Zeitschr. f. Biologie, **37**.

[4] Fr. Müller, Berl. klin. Wochenschr., 1887; Reale and Boeri, Maly's Jahresber., **24**; Harnack and Kleine, l. c.; Presch, Virchow's Arch., **119**; Yvon, Arch. de Physiol. (5), **10**.

[5] Benedict, Zeitschr. f. klin. Med., **36**; Harnack and Kleine, l. c.; Folin, Amer. Journ. of Physiol., **13**.

According to LÉPINE, a part of the neutral sulphur is more readily oxidized (directly with chlorine or bromine) into sulphuric acid than the other, which is only converted into sulphuric acid after fusing with potash and saltpeter. According to the investigations of W. SMITH,[1] it is probable that the most unoxidizable part of the neutral sulphur occurs as sulpho-acids. An increased elimination of neutral sulphur has been observed in various diseases, such as pneumonia, cystinuria, and especially where the flow of bile into the intestine is prevented.

The total quantity of sulphur in the urine is determined by fusing the solid urinary residue with saltpeter and caustic alkali. The quantity of neutral sulphur is determined as the difference between the total sulphur and the sulphur of the sulphate and ethereal-sulphuric acids. The readily oxidizable part of the neutral sulphur is determined by oxidation with bromine or potassium chlorate and hydrochloric acid (LÉPINE, JEROME[2]).

Sulphuretted hydrogen occurs in the urine only under abnormal conditions or as a decomposition product. This compound may be produced from the neutral sulphur of the organic substances of the urine by the action of certain bacteria (FR. MULLER, SALKOWSKI[3]). Other investigators have given *hyposulphites* as the source of the sulphuretted hydrogen. The occurrence of hyposulphites in normal human urine, which is asserted by HEFFTER, is disputed by SALKOWSKI and PRESCH.[4] Hyposulphites occur constantly in cat's urine and, as a rule, also in dog's urine.

Antoxyproteic acid is a nitrogenous acid containing sulphur which BONDZYNSKI, DOMBROWSKI, and PANEK[5] have isolated from human urine. The composition of the acid was: C 43.21, H 4.91, N 24.4, S 0.61, and O 26.33 per cent. A part of the sulphur can be split off by alkali. This acid is soluble in water, is dextrorotatory, and is precipitated only from concentrated solution by phosphotungstic acid. It does not give the protein color reactions, but gives EHRLICH'S diazo-reaction (see below). The salts with the alkalies, barium, calcium, and silver are soluble in water, and of these salts that with barium and, to a still higher degree, the silver salt are soluble with difficulty in alcohol. The free acid and its salts are precipitated by mercuric nitrate and acetate, and by this last reagent even from solutions strongly acidified with acetic acid. Basic lead acetate does not precipitate the pure acid.

Oxyproteic acid is the name given by BONDZINSKI and GOTTLIEB[6] to a nitrogenous acid containing sulphur and which they prepared from human urine, which has recently been further studied by BONDZYNSKI, DOMBROWSKI and PANEK. This acid contained C 39.62, H 5.64, N 18.08, S 1.12, and O 35.54 per cent, and also contains sulphur which could be split off. On cleavage it yields no tyrosine, nor does it give EHRLICH's diazo reaction, the xanthoproteic nor the biuret reaction. It gives a faint indication of a MILLON reaction and is not precipitated by phosphotungstic acid, hence it leads to an error in the PFLÜGER-BOHLAND's method for estimating urea. The acid soluble in water is precipitated by mercuric nitrate and acetate in neutral solutions, but is not precipitated by basic lead acetate. The salts of this acid are readily soluble in water and more soluble in alcohol than the corresponding salts of antoxyproteic acid.

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[1] Lépine, l. c.; Smith, Zeitschr. f. physiol. Chem., **17**.

[2] Jerome, Pflüger's Arch., **60**.

[3] Fr. Müller, Berlin. klin. Wochenschr., 1887; Salkowski, *ibid.*, 1888.

[4] Heffter, Pflüger's Arch., **38**; Salkowski, *ibid.*, **39**; Presch, Virchow's Arch., **119**.

[5] Zeitschr. f. physiol. Chem., **46**.

[6] Centralbl. f. d. med. Wissensch., 1897, No. 33.

The acid which is found in large quantities especially in the urine of dogs poisoned with phosphorus (BONDZYNSKI and GOTTLIEB) is considered like the preceding acids as an intermediary oxidation product of the proteins, and oxyproteic acid seems to represent a higher state of oxidation or a demolition of the proteins than the antoxyproteic acid.

The acid called *uroproteic acid* by CLOETTA is probably a mixture of several bodies, according to the recent investigations of BONDZYNSKI, DOMBROWSKI, and PANEK. The same appiles also to the barium oxyproteate prepared by PREGL [1] from the urine.

Alloxyproteic acid is a third acid related to the above, which was first isolated by BONDZYNSKI and PANEK [2] from the urine and then carefully studied with DOMBROWSKI. The composition is: C 41.33, H 5.70, N 13.55, S, 2.19, and O, 37.23 per cent, based upon new investigations. The free acid is soluble in water. It gives neither the biuret reaction nor Ehrlich's reaction, and is not precipitated by phosphotungstic acid. Differing from the other acids, it is precipitated by basic lead acetate and its salts are only slightly soluble in alcohol.

The preparation of the three above-mentioned acids is based in part upon the fact that alloxyproteic acid alone is precipitated by basic lead acetate and that the two other acids can be precipitated from the filtrate by mercuric acetate, the antoxyproteic acid in acetic acid solution, and the oxyproteic acid in neutral solution. The preparation is nevertheless very tedious and complicated and therefore we must refer to the original works [3] for details.

Uroferric acid is an acid isolated by THIELE [4] from the urine, according to SIEGFRIED's method for preparing pure peptone. It also contains sulphur, 3.46 per cent, and has the formula $C_{35}H_{56}N_8SO_{19}$. The acid forms a white powder which is readily soluble in water, saturated ammonium-sulphate solution, and methyl alcohol. It is soluble with difficulty in absolute alcohol, insoluble in benzene, chloroform, ether, and acetic ether. About one half of the sulphur can be split off as sulphuric acid on boiling with hydrochloric acid. The acid gives neither the biuret test nor Millon's or Adamkiewicz's reactions. It is precipitated by mercuric nitrate and sulphate, and also by phosphotungstic acid. This acid is hexabasic and its specific rotation is $(\alpha)_D^{18} = -32.5°$. On cleavage it yields melanine substances, sulphuric acid, aspartic acid, but no hexone bases. The existence of this acid is disputed by Bondzynski, Dombrowski and Panek.

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[1] Cloetta, Arch. f. exp. Path. u. Pharm., **40**; Pregl, Pflüger's Arch., **75**.

[2] Ber. d. d. chem. Gesellsch., **35**.

[3] Zeitschr. f. physiol. Chem., **46**.

[4] *Ibid.*, **37**.

Abderhalden and Pregl[1] have shown that human urine normally contains compounds which stand perhaps in close relationship to the polypeptides and which on hydrolysis with acids yield at least a part of the moities existing in the protein molecule. In the case investigated they obtained abundant glycocoll, also leucine, alanine, glutamic acid, phenyl-alanine, and probably also aspartic acid. The relationship between these polypeptide-like bodies and the above-mentioned proteic acids and to the uroferric acid has not been investigated.

Amino-acids may, when they are introduced in large amounts into the body, also pass in part into the urine. This has been shown for *r*-alanine by R. Hirsch for the dog, and by Plaut and Reese for dog and man, and for *r*-leucine by Abderhalden and Samuely[2] in rabbits. Embden and Reese, Forssner, Abderhalden and Schittenhelm, and Samuely[3] were able, by means of the naphthaline sulphochloride method, to detect glycocoll in normal human urine, and this glycocoll must occur in the urine in a combination which is readily split by alkali. Although we have numerous investigations, other amino-acids besides glycocoll could not be detected in normal human urine, while, on the contrary, in pathological conditions other amino-acids have been found several times. The amino-acid fraction of the urine seems to be increased in starvation and in high altitudes (Loewy[4]).

Organic combinations containing phosphorus (glycerophosphoric acid, phosphocarnic acid (Rockwood), etc., which yield phosphoric acid on fusing with saltpeter and caustic alkali, are also found in urine (Lépine and Eymonnet, Oertel). With a total elimination of about 2.0 grams total P_2O_5, Oertel found on an average about 0.05 gram P_2O_5 as phosphorus in organic combination. According to Symmers[5] the organic combined phosphoric acid may in many pathological conditions be 25–50 per cent of the total phosphoric acid. In lymphatic leucæmia, and especially in degenerative diseases of the nervous system, the quantity may increase.

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[1] Zeitschr. f. physiol. Chem., **46**.

[2] R. Hirsch, Zeitschr. f. exp. Path. u. Therap., **1**; Plaut and Reese, Hofmeister's Beiträge, **7**; Abderhalden and Samuely, Zeitschr. f. physiol. Chem., **47**.

[3] Embden and Reese, Hofmeister's Beiträge, **7**; G. Forssner, Zeitschr. f. physiol. Chem., **47**; Abderhalden and Schittenhelm, *ibid.*, **47**; Samuely, *ibid.*, **47**.

[4] Deutsch. med. Wochenschr., 1905.

[5] Rockwood, Arch. f. (Anat. u.) Physiol., 1895; Oertel, Zeitschr. f. physiol. Chem., **26**, which cites the other works. See also Keller, Zeitschr, f. physiol. Chem., **29**; Mandel and Oertel, N. Y. Univ. Bull. Med. Sciences, **1**; Symmers, Journ. of Path. and Bact., **10**.

Enzymes of various kinds have been isolated from the urine. Among these may be mentioned *pepsin* (**Brucke** and others), which, according to **Matthes**, undoubtedly originates from the stomach, and a *diastatic enzyme* (**Cohnheim** and others) and *trypsin*.[1]

Mucin. The nubecula consists, as shown by K. **Mörner**,[2] of a mucoid which contains 12.74 per cent N and 2.3 per cent S. This mucoid, which apprarently originates in the urinary passages, may pass to a slight extent into solution in the urine. In regard to the nature of the mucins and nucleoalbumins otherwise occurring in the urine we refer the reader to the pathological constituents of the urine.

Ptomaines and *leucomaines*, or poisonous substances of an unknown kind, which are often described as alkaloidal substances, occur in normal urine (**Pouchet**, **Bouchard**, **Aducco**, and others). Under pathological conditions the quantity of these substances may be increased (**Bouchard**, **Lépine** and **Guerin**, **Villiers**, **Griffiths**, **Albu**, and others). Within the last few years the poisonous properties of urine have been the subject of more thorough investigation, especially by **Bouchard**. He found that the night urine is less poisonous than the day urine, and that the poisonous constituents of the day and night urines have not the same action. In order to be able to compare the toxic power of the urine under different conditions, **Bouchard** determines the UROTOXIC COEFFICIENT, which is the weight of rabbit in kilos that is killed by the quantity of urine excreted in twenty-four hours by 1 kilo of the person experimented upon.[3]

Baumann and v. **Udránszky** have shown that ptomaines may occur in the urine under pathological conditions. They demonstrated the presence of the two ptomaines discovered and first isolated by **Brieger**—*putrescine*, $C_4H_{12}N_2$ (tetramethylendiamine), and *cadaverine*, $C_5H_{14}N_2$ (pentamethylendiamine)—in the urine of a patient suffering from cystinuria and catarrh of the bladder. Cadaverine has later been found by **Stadthagen** and **Brieger** in the urine in two cases of cystinuria. **Brieger**, v. **Udránszky** and **Baumann**, and **Stadthagen** have shown that neither these nor other diamines occur under physiological conditions, while **Dombrowski**, on the contrary, found cadaverine besides another ptomaine with the formula $C_2H_{15}NO_2$ in normal urines, and **Kutscher** and **Lohmann**[4] have found *neurine*. The occurrence in normal urine of any "*urine poison*" is denied by certain investigators, such as **Stadthagen**, **Beck**, and v. d. **Bergh**.[5] The poisonous action of the urine, according to them, is due in part to the potassium salts and in part to the sum of the toxicity of the other normal urinary constituents (urea, creatinine, etc.), which have very little poisonous action individually. The occurrence of special urine poisons under normal conditions is difficult to deny on account of numerous statements on this subject, although the chemical nature of these substances is still not sufficiently known.

Many substances have been observed in animal urine which are not found in human urine. To these belong the above-described *kynurenic acid*, *urocanic acid*, also found in dog's urine and which seems to stand in some relationship to the purine bases; *damaluric acid* and *damolic acid* (according to SCHOTTEN,[1] probably a mixture of benzoic acid with volatile fatty acids), obtained by the distillation of cow's urine; and lastly *lithuric acid*, found in the urinary concrements of certain animals.

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[1] In regard to the literature on enzymes in the urine, see Huppert-Neubauer, 599; Matthes, Arch. f. exp. Path. u. Pharm., **49**.

[2] Skand. Arch. f. Physiol., **6**.

[3] A complete bibliography on the ptomaines and leucomaines of the urine is found in Huppert-Neubauer, 403.

[4] Baumann and Udránszky, Zeitschr. f. physiol. Chem., **13**; Stadthagen and Brieger, Virchow's Arch., **115**; Dombrowski, Arch. polonais. d. sciences biol., 1903; Kutscher and Lohmann, Zeitschr. f. physiol. Chem., 48.

[5] Stadthagen, Zeitschr. f. klin. Med., **15**; Beck, Pflüger's Arch., **71**; v. d. Bergh, Zeitschr. f. klin. Med., **35**.

III. Inorganic Constituents of Urine.

Chlorides. The chlorine occurring in the urine is undoubtedly combined with the bases contained in this excretion; the chief part is in combination with sodium. In accordance with this, the quantity of chlorine in the urine is generally expressed as NaCl.

The question as to whether a part of the chlorine contained in the urine exists as organic combinations, as considered by BERLIOZ and LEPINOIS, is still disputed.[2]

The quantity of chlorine combinations in the urine is subject to considerable variation. In general the quantity from a healthy adult on a mixed diet is 10–15 grams of NaCl per twenty-four hours. The quantity of common salt in the urine depends chiefly upon the quantity of salt in the food, with which the elimination of chlorine increases and decreases. The free drinking of water also increases the elimination of chlorine, which is greater during activity than during rest (at night). Certain organic chlorine combinations, such as chloroform, may increase the elimination of inorganic chlorides by the urine (ZELLER, KAST[3]).

In diarrhœa, in quick formation of large transudates and exudates, also in specially marked cases of acute febrile diseases at the time of the crisis, the elimination of NaCl is materially decreased. The excretion of chlorine may vary considerably in disease, but still the NaCl taken with the food has here, as in physiological conditions, a great influence on the NaCl excretion.[4]

The *quantitative estimation of chlorine* in the urine is most simply performed by titration with silver-nitrate solution. The urine must not contain either proteid (which if present must be removed by coagulation) or iodine or bromine compounds.

In the presence of bromides or iodides evaporate a measured quantity of the urine to dryness, fuse the residue with saltpeter and soda, dissolve the fused mass in water, and remove the iodine or bromine by the addition of dilute sulphuric acid and some nitrite, and thoroughly shake with carbon disulphide. The liquid thus obtained may now be titrated with silver nitrate according to VOLHARD'S method. The quantity of bromide or iodide is calculated as the difference between the quantity of silver-nitrate solution used for the titration of the solution of the fused mass and the quantity used for the corresponding volume of the original urine.

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[1] Zeitschr. f. physiol. Chem., **7**.

[2] Berlioz and Lepinois, see Chem. Centralbl., 1894, **1**, and 1895, **1**; also Petit and Terrat, *ibid.*, 1894, **2**, and Vitali, *ibid.*, 1897, **2**; Viele and Moitessier, Maly's Jahresber., **31**; Meillère, *ibid.*; Bruno, *ibid.*, 452.

[3] Zeller, Zeitschr. f. physiol. Chem., 8; Kast, *ibid.*, **11**; Vitali, Chem. Centralbl., 1899, 2.

[4] On the elimination of chlorine in disease, see Albu and Neuberg, Physiol. u. Pathol. des Mineralstoffwechsels, Berlin, 1906.

The otherwise excellent titration method of MOHR, according to which we titrate with silver nitrate in neutral liquids, using neutral potassium chromate as an indicator, cannot be used directly on the urine in careful work. Organic urinary constituents are also precipitated by the silver salt, and the results are therefore somewhat high for the chlorine. If this method is to be employed, the organic urinary constituents must first be destroyed. For this purpose evaporate to dryness 5–10 c.c. of the urine, after the addition of 1 gram of chlorine-free soda and 1–2 grams chlorine-free saltpeter, and carefully fuse. The mass is dissolved in water, acidified faintly with nitric acid, and then neutralized exactly with pure calcium carbonate. This neutral solution is used for the titration.

The silver-nitrate solution may be a N/10 one. It is often made of such a strength that each cubic centimeter corresponds to 0.006 gram Cl or 0.01 gram NaCl. This last-mentioned solution contains 29.075 grams of $AgNO_3$ in 1 liter.

FREUND and TOEPFER, as well as BÖDTKER,[1] have suggested modifications of MOHR'S method.

VOLHARD'S METHOD. Instead of the preceding determination, VOLHARD'S method, which can be performed directly on the urine, may be employed. The principle is as follows: All the chlorine from the urine acidified with nitric acid is precipitated by an excess of silver nitrate, filtered, and in a measured part of the filtrate the quantity of silver added in excess is determined by means of a sulphocyanide solution. This excess of silver is completely precipitated by the sulphocyanide, and a solution of some ferric salt, which, as is well known, gives a blood-red reaction with the smallest quantity of sulphocyanide, is used as an indicator.

We require the following solutions for this titration: 1. A silver-nitrate solution which contains 29.075 grams of $AgNO_3$ per liter and of which each cubic centimeter corresponds to 0.01 gram NaCl or 0.00607 gram Cl. 2. A saturated solution at the ordinary temperature of chlorine-free iron alum or ferric sulphate. 3. Chlorine-free nitric acid of a specific gravity of 1.2. 4. A potassium-sulphocyanide solution which contains 8.3 grams KCNS per liter, and of which 2 c.c. corresponds to 1 c.c. of the silver-nitrate solution.

About 9 grams of potassium sulphocyanide is dissolved in water and diluted to 1 liter. The quantity of KCNS contained in this solution is determined by the silver-nitrate solution in the following way: Measure exactly 10 c.c. of the silver solution and treat it with 5 c.c. of nitric acid and 1–2 c.c. of the ferric-salt solution and dilute with water to about 100 c.c. Now the sulphocyanide solution is added from a burette, constantly stirring until a permanent faint-red coloration of the liquid takes place. The quantity of sulphocyanide found in the solution by this means indicates how much it must be diluted to be of the proper strength. Titrate once more with 10 c.c. of $AgNO_3$ solution and correct the sulphocyanide solution by the careful addition of water until 20 c.c. exactly corresponds to 10 c.c. of the silver solution.

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[1] Freund and Toepfer, see **Maly's Jahresber., 22; Bödtker, Zeitschr. f. physiol. Chem., 20.**

The determination of the chlorine in the urine is performed by this method in the following way: Exactly 10 c.c. of the urine is placed in a flask which has a mark corresponding to 100 c.c. and which is provided with a stopper; 5 c.c. of nitric acid is added; dilute with about 50 c.c. of water and then allow exactly 20 c.c. of the silver-nitrate solution to flow in. Close the flask with the stopper and shake well, remove the stopper and wash it with distilled water into the flask, and fill the flask to the 100-c.c. mark with distilled water. Close again with the stopper, carefully mix by shaking, and filter through a dry filter. Measure off 50 c.c. of the filtrate by means of a dry pipette, add 3 c.c. of ferric-salt solution, and allow the sulphocyanide solution to flow in until the liquid above the precipitate has a permanent red color. The calculation is very simple. For example, if 4.6 c.c. of the sulphocyanide solution was necessary to produce the final reaction, then for 100 c.c. of the filtrate (=10 c.c. urine) 9.2 c.c. of this solution is necessary. 9.2 c.c. of the sulphocyanide solution corresponds to 4.6 c.c. of the silver solution, and since $20-4.6=15.4$ c.c. of the silver solution was necessary to completely precipitate the chlorine in 10 c.c. of the urine, then 10 c.c. contains 0.154 gram of NaCl. The quantity of sodium chloride in the urine is therefore 1.54 per cent, or 15.4 p. m. If we always use 10 c.c. for the determination, and always 20 c.c. of $AgNO_3$ solution, and dilute with water, to 100 c.c., the quantity of NaCl in 1000 parts of the urine is found by subtracting from 20 the number of cubic centimeters of sulphocyanide (R) required with 50 c.c. of the filtrate. The quantity of NaCl p. m. therefore under these circumstances $=20-R$, and the percentage of $NaCl=\frac{20-R}{10}$.

If it is necessary to destroy the organic urinary constituents before titration, this can best be performed, according to DEHN,[1] by evaporating the urine (10 c.c.), after the addition of a small amount of sodium peroxide, to dryness on the water-bath then faintly acidifying with nitric acid and then titrating according to VOLHARD. Incineration is unnecessary.

For the approximate estimation of chlorine in the urine EKEHORN has made use of VOLHARD's titration method by using for the determination a glass tube closed at one end and divided into half cubic-centimeters and called the chlorometer. The reagents necessary are: (*a*) a mixture of 20 c.c. silver-nitrate solution (according to VOLHARD), 5 c.c. nitric acid and water to 100 c.c.; (*b*) 40 c.c. sulphocyanide solution (according to VOLHARD) and 60 c.c. of a ferric alum, chlorine-free and saturated at the ordinary temperature. The silver-nitrate solution, of which each cubic centimeter corresponds to 0.002 gm. NaCl, is equivalent to the iron sulphocyanide solution. First 2 c.c. of the urine is placed in the graduated tube and then 0.5 c.c. sulphocyanide solution, and the silver-nitrate solution gradually added (shaking the tube closed with a rubber stopper) until the coloration of the sulphocyanide just disappears. 0.5 c.c. is subtracted from the silver solution for the 0.5 c.c. of the sulphocyanide; the tube is so graduated that the quantity of NaCl in the urine in parts per thousand is read off directly on the tube. The difference between these results and those obtained by VOLHARD'S titration method amounts only, according to C. TH. MÖRNER,[1] to 0.25 to at most 0.5 p. m.

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[1] Zeitschr. f. physiol. Chem., **44**.

The approximate estimation of chlorine in the urine (which must be free from proteid) is made by strongly acidifying with nitric acid and then adding to it, drop by drop, a concentrated silver-nitrate solution (1:8). In a normal quantity of chlorides the drop sinks to the bottom as a rather compact cheesy lump. In diminished quantities of chlorides the precipitate is less compact and coherent, and in the presence of very little chlorine a fine white precipitate or only a cloudiness or opalescence is obtained.

Phosphates. Phosphoric acid occurs in acid urines partly as dihydrogen, MH_2PO_4, and partly as monohydrogen M_2HPO_4, phosphates, both of which may be found in acid urines at the same time. OTT[2] found that on an average 60 per cent of the total phosphoric acid was di- and 40 per cent was monohydrogen phosphate. The total quantity of phosphoric acid is very variable and depends on the character and the quantity of food. The average quantity of P_2O_5 is in round numbers 2.5 grams, with a variation of 1-5 grams per day. A small part of the phosphoric acid of the urine originates from the burning of organic compounds, such as nuclein, protagon, and lecithin, within the organism; on exclusive feeding with substances rich in nuclein or pseudonuclein the quantity of phosphates is essentially increased; still it is undecided to what extent the excretion of phosphoric acid is a measure of the absorption and decomposition of these bodies.[3] The greater part originates from the phosphates of the food, and the quantity of phosphoric acid eliminated is greater when the food is rich in alkali phosphates in proportion to the quantity of lime and magnesium phosphates. If the food contains much lime and magnesia, large quantities of earthy phosphates are eliminated by the excrement; and even though the food contains considerable amounts of phosphoric acid in these cases, the quantity excreted by the urine is small. This is true especially of herbivora, in which the kidneys are the chief organs for the excretion of alkali phosphates. In man, according to EHRSTRÖM, the content of lime in the food seems to play no important rôle, as in his experiments about one-half of the phosphoric acid taken as $CaHPO_4$ was absorbed; still the extent of phosphoric-acid excretion through the urine depends in man not only upon the total quantity of phosphoric acid in the food, but also upon the relative amounts of the alkaline earths and the alkali salts of the food. In carnivora, in which phosphate injected subcutaneously is eliminated by the intestine (BERGMANN), the urine is habitually poor in phosphates.[1]

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[1] Ekehorn, Hygiea, Stockholm, 1906; Mörner, Upsala Läkaref. Förh. (N. F.), **11**.

[2] Zeitschr. f. physiol. Chem., **10**.

[3] See A. Gumlich, Zeitschr. f. physiol. Chem., **18**; Roos, *ibid.*, **21**; Weintraud, Arch. f. (Anat. u.) Physiol., 1895; Milroy and Malcolm, Journ. of Physiol., **23**; Röhmann and Steinitz, Pflüger's Arch., **72**; Loewi, Arch. f. exp. Path. u. Pharm., **44** and **45**.

As the extent of the elimination of phosphoric acid is mostly dependent upon the character of the food and the absorption of the phosphates in the intestine, it is apparent that the relationship between the nitrogen and phosphoric-acid excretion cannot run parallel. This is in fact so, and, according to EHRSTRÖM, the organism has the power of accumulating large quantities of phosphorus for a relatively long time independent of the condition of the nitrogen balance. With a certain regular food the relationship between nitrogen and phosphoric acid in the urine can be kept nearly constant. Thus on feeding with an exclusive meat diet, as observed by VOIT[2] in dogs, when the nitrogen and phosphoric acid (P_2O_5) of the food exactly reappeared in the urine and fæces, the relationship was 8.1:1. In starvation, as shown by the compilation of R. TIGERSTEDT,[3] the phosphorized constituents of the body are destroyed to a much greater extent than when food is given very poor in phosphorus. In starvation this relationship is changed, namely, relatively more phosphoric acid is eliminated, which seems to indicate that besides flesh and related tissues another tissue rich in phosphorus is largely destroyed. The starvation experiments show that this is the bone-tissue. According to PREYSZ, OLSAVSZKY, KLUG, and I. MUNK[4] the elimination of phosphoric acid is considerably increased by intense muscular work.

As the phosphoric acid is in part derived from the nucleins it would be expected that in those diseases in which the excretion of alloxuric bodies was increased the phosphoric acid would also be augmented. This is not the case, and indeed we have observed cases with an increased elimination of alloxuric bodies with a diminution in the phosphoric-acid excretion. Cases of leucæmia have been observed in which the phosphoric-acid excretion was reduced, although there was a pronounced increase in the number of leucocytes. In these cases there may be a subsequent excretion or retention of phosphoric acid. This last condition occurs also in inflammatory and renal diseases. The earthy phosphates of the urine sometimes have the tendency of precipitating either spontaneously or after warming, and this has been called *phosphaturia*. We are dealing here with a diminished acidity and, it seems, with a diminished excretion of phosphoric acid and an increased elimination of lime, or at least an essentially different relationship between the phosphoric acid and the alkaline earths of the urine, as compared with the normal (PANEK, IWANOFF, SOETBER and KRIEGER).[1]

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[1] Ehrström, Skand. Arch. f. Physiol., **14**; Bergmann, Arch. f. exp. Path. u. Pharm., **47**.

[2] Physiologie des allgemeinen Stoffwechsels und der Ernährung in L. Hermann's Handbuch, **6**, Thl. 1, 79.

[3] Skand. Arch. f. Physiol., **16**.

[4] Preysz, see Maly's Jahresber., **21**; Olsavszky and Klug, Pflüger's Arch., **54**; Munk. Arch. f. (Anat. u.) Physiol., 1895.

Quantitative Estimation of the Total Phosphoric Acid in the Urine. This estimation is most simply performed by titrating with a solution of uranium acetate. The principle of the titration is as follows: A warm solution of phosphates containing free acetic acid gives a whitish-yellow precipitate of uranium phosphate with a solution of a uranium salt. This precipitate is insoluble in acetic acid, but dissolves in mineral acids. and on this account there is always added, in titrating, a certain quantity of sodium-acetate solution. Potassium ferrocyanide is used as the indicator, which does not act on the uranium-phosphate precipitate, but gives a reddish-brown precipitate or coloration in the presence of the smallest amount of soluble uranium salt. The solutions necessary for the titration are: 1. A solution of a uranium salt of which each cubic centimeter corresponds to 0.007 gram P_2O_5 and which conains 20.3 grams of uranium oxide per liter. 20 c.c. of this solution corresponds to 0.100 gram P_2O_5. 2. A solution of sodium acetate. 3. A freshly prepared solution of potassium ferrocyanide.

The uranium solution is prepared from uranium nitrate or acetate. Dissolve about 35 grams uranium acetate in water, add some acetic acid to facilitate solution, and dilute to 1 liter. The strength of this solution is determined by titrating with a solution of sodium phosphate of known strength (10.085 grams crystallized salt in 1 liter, which corresponds to 0.100 gram P_2O_5 in 50 c.c.). Proceed in the same way as in the titration of the urine (see below), and correct the solution by diluting with water, and titrate again until 20 c.c. of the uranium solution corresponds exactly to 50 c.c. of the above phosphate solution.

The sodium-acetate solution should contain 10 grams sodium acetate and 10 grams conc. acetic acid in 100 c.c. For each titration 5 c.c. of this solution is used with 50 c.c. of the urine.

In performing the titration, mix 50 c.c. of filtered urine in a beaker with 5 c.c. of the sodium acetate, cover the beaker with a watch-glass, and warm over the water-bath. Then allow the uranium solution to flow in from a burette, and when the precipitate does not seem to increase, place a drop of the mixture on a porcelain plate with a drop of the potassium-ferrocyanide solution. If the amount of uranium solution added has not been sufficient, the color will remain pale yellow and more uranium solution must be added; but as soon as the slightest excess of uranium solution has been used the color becomes a faint reddish brown. When this point has been obtained, warm the solution again and add another drop. If the color remains of the same intensity, the titration is ended; but if the color varies, add more uranium solution, drop by drop, until a permanent coloration is obtained after warming, and now repeat the test with another 50 c.c. of the urine. The calculation is so simple that it is unnecessary to give an example.

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[1] Panek, see Maly's Jahresber., **30**, 112; Iwanoff, Biochem. Centralbl., **1**, 710; Soetber and Krieger, Deutsch. Arch. f. klin. Med., **72**; Campani, Biochem. Centralbl., **3**, 616; Tobler, Arch. f. exp. Path. u. Pharm., **52**.

In the above manner one determines the total quantity of phosphoric acid in the urine. If we wish to know the phosphoric acid combined with alkaline earths or with alkalies, we first determine the total phosphoric acid in a portion of the urine and then remove the earthy phosphates in another portion by ammonia. The precipitate is collected on a filter, washed, transferred into a beaker with water, treated with acetic acid, and dissolved by warming. This solution is now diluted to 50 c.c. with water, and 5 c.c. sodium-acetate solution added, then titrated with uranium solution. The difference between the two determinations gives the quantity of phosphoric acid combined with the alkalies. The results obtained are not quite accurate, as a partial transformation of the monophosphates of the alkaline earths and also calcium diphosphate into triphosphates of the alkaline earths and ammonium phosphate takes place on precipitating with ammonia, and the method gives too high results for the phosphoric acid combined with alkalies and remaining in solution.

Sulphates. The sulphuric acid of the urine originates only to a very small extent from the sulphates of the food. A disproportionately greater part is formed by the burning within the body of the proteins which contain sulphur, and it is chiefly this formation of sulphuric acid from the proteins which gives rise to the previously mentioned excess of acids over the bases in the urine. The quantity of sulphuric acid eliminated by the urine amounts to about 2.5 grams H_2SO_4 per day. As the sulphuric acid chiefly originates from the proteins, it follows that the elimination of sulphuric acid and the elimination of nitrogen runs nearly parallel, and the relationship $N:H_2SO_4$ is about 5:1. A complete parallelism can hardly be expected, as in the first place a part of the sulphur is always eliminated as neutral sulphur, and secondly because the small proportion of sulphur in different protein bodies undergoes greater variation as compared with the large proportion of nitrogen contained therein. In general the elimination of nitrogen and sulphuric acid under normal and under diseased conditions seem to run rather parallel. Sulphuric acid occurs in the urine partly preformed (sulphate-sulphuric acid) and partly as ethereal, sulphuric acid. The first is designated as *A*- and the other as *B*-sulphuric acid.

The *quantity of total sulphuric acid* is determined in the following way, but at the same time the precautions described in other works must be observed. 100 c.c. of filtered urine is treated with 5 c.c. of concentrated hydrochloric acid and boiled for fifteen minutes. While boiling precipitate with 2 c.c. of a saturated $BaCl_2$ solution, and warm for a little while until the barium sulphate has completely settled. The precipitate must then be washed with water and also with alcohol and ether (to remove resinous substances), and then treated according to the usual method.

The separate determination of the sulphate-sulphuric acid and the ethereal-sulphuric acid may be accomplished, according to BAUMANN's method, by first precipitating the sulphate-sulphuric acid by $BaCl_2$ from the urine acidified with acetic acid, then decomposing the ethereal-sulphuric acid by boiling after the addition of hydrochloric acid, and finally determining the sulphuric acid set free as barium sulphate. A still better method is the following, suggested by SALKOWSKI:[1]

200 c.c. of urine is precipitated by an equal volume of a barium solution which consists of 2 vols. barium hydrate and 1 vol. barium-chloride solution, both saturated at the ordinary temperature. Filter through a dry filter, measure off 100 c.c. of the filtrate which contains only the ethereal-sulphuric acid, treat with 10 c.c. of hydrochloric acid of a specific gravity 1.12, boil for fifteen minutes, and then warm on the water-bath until the precipitate has completely settled and the supernatant liquid is entirely clear. Filter and wash with warm water and with alcohol and ether, and proceed according to the generally prescribed method. The difference between the ethereal-sulphuric acid found and the total quantity of sulphuric acid as determined in a special portion of urine is taken to be the quantity of sulphate-sulphuric acid.

FOLIN[2] has suggested a method for estimating the sulphate-sulphuric acid as well as the ethereal-sulphuric acid, and also the total sulphur, which is somewhat different from the ordinary methods.

Nitrates occur in small quantities in human urine (SCHÖNBEIN), and they probably originate from the drinking-water and the food. According to WEYL and CITRON,[3] the quantity of nitrates is smallest with a meat diet and greatest with vegetable food. The average amount is about 42.5 milligrams per liter.

Potassium and Sodium. The quantity of these bodies eliminated by the urine by a healthy adult on a mixed diet is, according to SALKOWSKI,[4] 3–4 grams K_2O and 5–6 grams Na_2O, with an average of about 2–3 grams K_2O and 4–6 grams Na_2O. The proportion of K to Na is ordinarily 3:5. The quantity depends above all upon the food. In starvation the urine may become richer in potassium than in sodium, which results from the lack of common salt and the destruction of tissue rich in potassium. The quantity of potassium may be relatively increased during fever, while after the crisis the reverse is the case.

The quantitative estimation of these bodies is performed by the gravimetric methods as described in works on quantitative analysis. In the determination of the total alkalies recently new methods have been devised by PRIBRAM and GREGOR, and for the potassium alone a method by AUTENRIETH and BERNHEIM.[5]

Ammonia. Some ammonia is habitually found in human urine and in that of carnivora. As above stated (page 551), on the formation of urea from ammonia, this quantity may represent the small amount of ammonia which. is excluded from the synthesis to urea by being combined with acids formed in excess by combustion and not united with the fixed alkalies. This view is confirmed by the observations of CORANDA, who found that the elimination of ammonia was smaller on a vegetable diet and larger on a rich meat diet than on a mixed diet. On a mixed diet the average amount of ammonia eliminated by the urine is about 0.7 gram NH_3 per day (NEUBAUER), corresponding to 4.6–5.6 per cent of the total nitrogen of the urine according to CAMERER, Jr. As above stated, all the ammonia of the urine is not represented by the residue which has eluded synthesis into urea by neutralization with acids, becaues, as shown by STADELMANN and BECKMANN,[1] ammonia is eliminated by the urine even during the continuous administration of fixed alkalies.

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[1] Baumann, Zeitschr. f. physiol. Chem., **1**; Salkowski, Virchow's Arch., **79**.

[2] Journ of Biol. Chem., **1**, and Amer. Journ. of Physiol., **13**.

[3] Schönbein, Journ. f. prakt. Chem., **92**; Weyl, Virchow's Arch., **96**, with Citron, *ibid.*, 101.

[4] *Ibid.*, **53**.

[5] Pribram and Gregor, Zeitschr. f. analyt. Chem., **38**; Autenrieth and Bernheim, Zeitschr f. physiol. Chem., **37**.

Ammonia exists on an average of about 0.90 milligram in 100 c.c. of human blood, and in different amounts in all the tissues thus far investigated.[2] According to NENCKI and ZALESKI[3] it is abundantly formed in the cells of the digestive glands, the stomach, the pancreas, and the intestinal mucosa (of dogs) at the time when protein foods are being digested and transported to the liver. As the ammonia introduced into the liver is transformed into urea (see above), we can therefore expect that in certain diseases of the liver an increased elimination of ammonia and a decreased excretion of urea will occur. In how far this is true has already been stated (page 554), and we refer to the researches of the various authors there cited.

In man and certain animals the elimination of ammonia is increased by the introduction of mineral acids; and, as shown by JOLIN,[4] organic acids, such as benzoic acid, which are not destroyed in the body act in a similar manner. The ammonia set free in the protein destruction is in part used in the neutralization of the acids introduced, and in this way a destructive removal of fixed alkalies is prevented. This unequal behavior of different animals towards acidosis has been discussed in the previous pages.

Acids formed in the destruction of proteins in the body act on the elimination of ammonia like those introduced from without. For this reason the quantity of ammonia in human urine is increased under such conditions and in such diseases where an increased formation of acid takes place because of an increased metabolism of proteins. This is the case with a lack of oxygen in fevers and diabetes. In the last-mentioned disease organic acids, β-oxybutyric acid and acetoacetic acid, are produced, which pass into the urine combined with ammonia.[1] Other observations also indicate that the elimination of ammonia by the urine is increased on insufficient or diminished supply of alkalies or alkaline earths.

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[1] Coranda, Arch. f. exp. Path. u. Pharm., **12**; Stadelmann (and Beckmann). Einfluss der Alkalien auf den Stoffwechsel, etc. Stuttgart, 1890; Camerer, Zeitschr. f. Biologie, **43**.

[2] See Salaskin, Zeitschr f. physiol. Chem., **25**, 449, and foot-notes 1 and 2, page 241.

[3] Arch. des science biol. de St. Pétersbourg, **4**, and Salaskin, l. c. See also Nencki and Zaleski, Arch. f. exp. Path. u. Pharm., **37**.

[4] Jolin, Skand. Arch. f. Physiol., **1**. In regard to the behavior of ammonium salts in the animal body, see Rumpf and Kleine, Zeitschr. f. Biologie, **34**, and the works cited on page 554.

The detection and quantitative estimation of ammonia used to be performed according to the method suggested by SCHLÖSING. The principle of this method is that the ammonia from a measured amount of urine is set free by lime-water in a closed vessel and absorbed by a measured amount of N/10 sulphuric acid. After the absorption of the ammonia the quantity is determined by titrating the remaining free sulphuric acid with a N/10 caustic-alkali solution. This method gives low results, and in exact work we must proceed as suggested by BOHLAND.[2]

The recent methods for estimating the ammonia are all based upon the distillation of the ammonia, after the addition of lime, magnesia, or alkali carbonate, at low temperatures either by the aid of vacuum (NENCKI and ZALESKI, WURSTER, KRÜGER, REICH, and SCHITTENHELM, and SCHAFFER) or by the aid of a current of air (FOLIN) and then collecting it in a standard acid.

According to the methods suggested by KRÜGER, REICH and SCHITTENHELM[3] 25 c.c. of the urine is placed in a distillation-flask with about 10 grams of NaCl and 1 gram of Na_2CO_3, and this distilled at 43° C. and a pressure of 30–40 millimeters Hg with the aid of an air-pump. Alcohol is added to prevent foaming. The ammonia is absorbed in N/10 acid contained in a PELIGOT tube surrounded by ice-water, and when the distillation is finished the acid is retitrated, making use of rosolic acid as indicator. In regard to details, see the original publications. SCHAFFER'S method is practically the same.

Calcium and magnesium occur in the urine chiefly as phosphates. The quantity of earthy phosphates eliminated daily is somewhat more than 1 gram, and of this amount ⅔ is magnesium and ⅓ calcium phosphate. This statement, as found by RENWALL and GROSS,[4] is not correct, or at least is not valid in general, as they found more calcium than magnesium in the urine. In acid urines the mono- as well as the dihydrogen earthy phosphates are found, and the solubility of the first, among which the calcium salt $CaHPO_4$ is especially insoluble, is particularly augmented by the presence in the urine of dihydrogen alkali phosphates and sodium chloride (OTT[5]). The quantity of alkaline earths in the urine depends on the composition of the food. The lime-salts absorbed are in great part excreted again into the intestine, and the quantity of lime-salts in the urine is therefore no measure of the absorption of the same. The introduction of readily soluble lime-salts or the addition of hydrochloric acid to the food may therefore cause an increase in the quantity of lime in the urine, while the reverse takes place on adding alkali phosphate to the food. Nothing is known with positiveness in regard to the constant and regular change in the elimination of calcium and magnesium salts in disease,[1] and in these conditions the excretion is chiefly dependent upon the diet, and upon the formation and introduction of acid.

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[1] On the elimination of ammonia in disease, see the recent works of Rumpf, Virchow's Arch., **134**; Hallervorden, *ibid.*

[2] Pflüger's Arch., **43**, 32

[3] Zeitschr. f physiol. Chem., **39**; Schaffer, Amer. Journ. of Physiol., **8**, which contains the literature.

[4] Renwall, Skand. Arch. f. Physiol, **16**; Gross, Biochem Centralbl., **4**, 189.

[5] Zeitschr. f. physiol. Chem., **10**

The quantity of calcium and magnesium is determined according to the ordinary well-known methods.

Iron occurs in the urine only in small quantities, and, as it seems from the investigations of KUNKEL, GIACOSA, KOBERT and his pupils, it does not exist as a salt, but as an organic combination—in part as pigment or chromogen. The statements in regard to the iron present seem to show that the quantity is very variable, from 1 to 11 milligrams per liter of urine (MAGNIER, GOTTLIEB, KOBERT and his pupils). JOLLES found as an average for twelve persons 8 milligrams of iron in twenty-four hours, while HOFFMANN, NEUMANN and MAYER [2] found lower results—an average of 1.09 and 0.983 milligrams. The quantity of *silicic acid* is ordinarily stated to amount to about 0.3 p. m. Traces of *hydrogen peroxide* also occur in the urine.

The *gases* of the urine are carbon dioxide, nitrogen, and traces of oxygen. The quantity of nitrogen is not quite 1 vol. per cent. The carbon dioxide varies considerably. In acid urines it is hardly one half as great as in neutral or alkaline urines.

IV. The Quantity and Quantitative Composition of Urine.

The quantity and composition of urine is liable to great variation. The circumstances which under physiological conditions exercise a great influence are the following: the blood-pressure, and the rapidity of the blood-current in the glomeruli; the quantity of urinary constituents, especially water in the blood; and, lastly, the condition of the secretory glandular elements. Above all, the quantity and concentration of the urine depend on the quantity of water which is introduced into the blood or which leaves the body in other ways. The excretion of urine is increased by drinking freely or by reducing the quantity of water otherwise removed; and it is decreased by a diminished ingestion of water or by a greater loss of water in other ways. Ordinarily in man just as much water is eliminated by the kidneys as by the skin, lungs, and intestine together. At lower temperatures and in moist air, since under these conditions the elimination of water by the skin is diminished, the excretion of urine may be considerably increased. Diminished introduction of water or increased elimination of water by other ways—as in violent diarrhœa or vomiting, or in profuse perspiration—greatly diminishes the amount of urine excreted. For example, the urine may sink as low as 500–400 c.c. per day in intense summer heat, while after copious draughts of water the elimination of 3000 c.c. of urine has been observed during the same time. The quantity of urine voided in the course of twenty-four hours varies considerably from day to day, the average being ordinarily calculated as 1500 c.c. for healthy adult men and 1200 c.c. for women. The minimum elimination occurs during the early morning, between 2 and 4 o'clock; the maximum, in the first hours after waking and from 1–2 hours after a meal.

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[1] See Albu and Neuberg, l. c.

[2] Kunkel, cited from Maly's Jahresber., **11**; Giacosa, *ibid.*, **16**; Kobert, Arbeiten des Pharm. Inst. zu Dorpat, **7**; Magnier, Ber, d. deutsch. chem. Gesellsch., **7**; Gottlieb, Arch. f. exp. Path. u. Pharm., **26**; Jolles, Zeitschr. f. anal. Chem., **36**; Hoffmann, Zeitschr. f. anal. Chem., **40**; Neumann and Mayer, Zeitschr. f. physiol. Chem., **37**.

The quantity of solids excreted per day is nearly constant, even though the quantity of urine may vary, and it is quite constant when the manner of living is regular. Therefore the percentage of solids in the urine is naturally in inverse proportion to the quantity of urine. The average amount of solids pert wenty-four hours is calculated as 60 grams. The quantity may be calculated with approximate accuracy from the specific gravity if the second and third decimals of this factor be multiplied by HÄSER's coefficient, 2.33. The product gives the amount of solids in 1000 c.c. of urine, and if the quantity of urine eliminated in twenty-four hours be measured, the quantity of solids in twenty-four hours may be easily calculated. For example, 1050 c.c. of urine of a specific gravity 1.021 was eliminated in twenty-four hours; therefore the quantity of solids excreted was $21\times2.33=48.9$ and $\frac{48.9\times1050}{1000}=51.35$ grams. LONG[1] has made a new determination of the coefficient for a specific gravity taken at 25° C. and finds that it is equal to 2.6, which corresponds nearly to HÄSER's coefficient at 15° C.

Those bodies which, under physiological conditions, affect the density of the urine are common salt and urea. The specific gravity of the first is 2.15 and the last only 1.32, so it is easy to understand, when the relative proportion of these two bodies essentially deviates from the normal, why the above calculation from the specific gravity is not exact. The same is true when a urine poor in normal constituents contains large amounts of foreign bodies, such as albumin or sugar.

As above stated, the percentage of solids in the urine generally decreases with a greater elimination, and a very considerable excretion of urine

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[1] Journ. Amer. Chem. Soc., **25**.

(*polyuria*) has therefore, as a rule, a lower specific gravity. An important exception to this rule is observed in urine containing sugar (*diabetes mellitus*), in which there is a copious excretion with a very high specific gravity due to the sugar. In cases where very little urine is excreted (*oliguria*), e.g., during profuse perspiration, in diarrhœa, and in fevers, the specific gravity of the urine is as a rule very high: the percentage of solids is also high and the urine has a dark color. Sometimes, as for example in certain cases of albuminuria, the urine may have a low specific gravity notwithstanding the oliguria, and be poor in solids and light in color.

In certain cases it is interesting to know the relationship between the carbon and the nitrogen, or the quotient C/N. This factor may vary between 0.7 and 1; as a rule, it amounts on an average to 0.87, but changes according to the nature of the food and is higher after a diet rich in carbohydrates than after food rich in fat (PREGL, TANGL, LANGSTEIN and STEINITZ, and others[1]).

It is difficult to give a tabular view of the composition of urine on account of its variation. For certain purposes the following table may be of some value, but it must not be overlooked that the results are not given for 1000 parts of urine, but only approximate figures for the quantities of the most important constituents which are eliminated during the course of twenty-four hours in a volume of 1500 c.c. of urine. These figures apply only to a diet which corresponds to VOIT's standard figures, namely 118 grams protein, 56 grams fat, and 500 grams carbohydrate per day, and to a man of average weight.

Daily quantity of solids = 60 grams.

Organic constituents = 35 grams.		Inorganic constituents = 25 grams.	
Urea . .	30.0 grams.	Sodium chloride (NaCl) . . .	15.0 grams.
Uric acid . . .	0.7 "	Sulphuric acid (H_2SO_4) . . .	2.5 "
Creatinine . .	1.5 "	Phosphoric acid (P_2O_5) . . .	2.5 "
Hippuric acid	0.7 "	Potash (K_2O)	3.3 "
Remaining organic bodies .	2.1 "	Ammonia (NH_3)	0.7 "
		Magnesia (MgO) Lime (CaO)	0.8 "
		Remaining inorganic bodies	0.2 "

Urine contains on an average 40 p. m. solids. The quantity of urea is about 20 p. m., and common salt about 10 p. m.

The physico-chemical methods are being used in urinary analysis even to a greater extent than in the analysis of other animal fluids. A great number of cryoscopic determinations but fewer conductivity determinations have been made. A constant relationship between the values found by physico-chemical methods and the analytical methods has been sought, for example between the freezing-point depression and the specific gravity or the common salt and others; or attempts have been made to find certain constants in the composition of the urine based upon the results of various methods, and in this way to obtain an explanation as to the mechanism of the excretion of urine in order to apply them for diagnostic purposes. The results obtained are, as is to be expected, so variable and dependent upon so many conditions which cannot be controlled that definite conclusions must be drawn with the greatest caution. In regard to the value and usefulness of the various constants and relations which are based upon theoretical considerations, the views are unfortunately still too divergent.

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[1] Pregl, Pflüger's Arch., 75, which contains the older literature; Tangl, Arch. f. (Anat. u.) Physiol., 1899, Suppl.; Langstein and Steinitz, Centralbl. f. Physiol., 19.

V. Casual Urinary Constituents.

The casual appearance in the urine of medicinal agents or of urinary constituents resulting from the introduction of foreign substances into the organism is of practical importance, because such compounds may interfere in certain urinary investigations; they also afford a good means of determining whether certain substances have been introduced into the organism or not. From this point of view a few of these bodies will be spoken of in a following section (on the pathological urinary constituents). The presence of these foreign bodies in the urine is of special interest in those cases in which they serve to elucidate the chemical transformations which certain substances undergo within the organism. As inorganic substances generally leave the body unchanged,[1] they are of very little interest from this standpoint; but the changes which certain organic substances undergo when introduced into the animal body may be studied by the transformation products as found in the urine.

The bodies belonging to the **fatty series** undergo, though not without exceptions, a combustion leading towards the final products of metabolism; still, often a greater or smaller part of the bodies in question escape oxidation and appear unchanged in the urine. A part of the acids belonging to this series, which are otherwise decomposed into water and carbonates and render the urine neutral or alkaline, may act in this manner. The *volatile fatty acids* poor in carbon are less easily oxidized than those rich in carbon, and they therefore pass unchanged into the urine in large amounts. This is especially true of formic and acetic acids (SCHOTTEN, GRÉHANT and QUINQUAUD[2]). The statements in regard to oxalic acid are contradictory. In birds, according to GAGLIO and GIUNTI, it is not oxidized. In mammals it is in great part oxidized, according to GIUNTI, while GAGLIO and POHL claim that it is not destroyed. MARFORI and GIUNTI claim that in human beings oxalic acid is in great part oxidized, although the recent investigations of SALKOWSKI, PIERALLINI, STRADOMSKY, KLEMPERER and TRITSCHLER [1] seem to show that the acid is only in part destroyed in the animal body. In order to exactly determine that portion of the ingested oxalic acid which is absorbed and excreted by the urine or oxidized in the body, it must necessarily be known whether or not a portion of the acid is destroyed in the intestine and is therefore not absorbed. Tartaric acids act differently, according to BRION; [2] thus in dogs the levotartaric acid is nearly entirely consumed, while a little more than 70 per cent of dextrotartaric acid is burnt. Racemic acid is oxidized to a still less extent in the animal body. Succinic and malic acids are completely combustible, according to POHL.[3] Examples of the different behavior of stereoisomeric substances have already been given on page 109.

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[1] In regard to the behavior of certain of these bodies, see Heffter, Die Ausscheidung körperfremden Substanzen im Harn, Ergebnisse d. Physiol., 2, Abt. 1.

[2] Schotten, Zeitschr. f. physiol. Chem., 7; Gréhant and Quinquaud, Compt.-rend., 104.

The *acid amides* appear not to be altered in the body (SCHULTZEN and NENCKI [4]). The *amino-acids* may indeed, when introduced into the body in large quantities, be in part eliminated unchanged by the urine; but otherwise, as stated above (page 550) for *leucine*, *glycocoll*, and *aspartic acid*, they are decomposed within the body, and may therefore cause an increased excretion of urea. That in the demolition of the amino-acids a deamidation takes place is shown by alanine yielding lactic acid and diaminopropionic acid, yielding glyceric acid, as mentioned in a previous chapter (VIII). The amino-acids give an instructive example of the unequal behavior of stereometric substances in the animal body, as the inactive acids are so changed and transformed that the component foreign to the body is more or less abundantly excreted, while that occurring in the body protein is oxidized (SCHITTENHELM and KATZENSTEIN, WOHLGEMUTH [5]). In connection with the amino-acids it is to be recalled that according to the observations of ABDERHALDEN and BERGELL [6] glycylglycine introduced subcutaneously in rabbits appeared in the urine as glycocoll.

Various amino acids show a somewhat different behavior. *Sarcosine*

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[1] Gaglio, Arch. f. exp. Path. u. Pharm., **22**; Giunti, Chem. Centralbl., 1897, **2**; Marfori, Maly's Jahresber., **20** and **27**; Pohl, Arch. f. exp. Path. u. Pharm., **37**; Salkowski, Berl. klin. Wochenschr., 1900; Pierallini, Virchow's Arch., **160**; Stradomsky, *ibid.*, **163**; Klemperer and Tritschler, Zeitschr. f. klin. Med., **44**.

[2] Zeitschr. f. physiol. Chem., **25**.

[3] Pohl, Arch. f. exp. Path. u. Pharm., **37**, which also contains the statements on the intermediary products formed in the oxidation of the fatty bodies.

[4] Zeitschr. f. Biologie, 8.

[5] Schittenhelm and Katzenstein, Zeitschr. f. exp. Path., **2**, cited from Biochem. Centralbl., **5**; Wohlgemuth, Ber. d. d. chem. Gesellsch., **38**.

[6] Zeitschr. f. physiol. Clem., **39**.

(methylglycocoll), $(CH_3)NH.CH_2.COOH$, which is not readily burnt, passes therefore in great part unchanged into the urine, but perhaps also passes in small part into the corresponding uramino-acid, *methylhydantoic acid,* $NH_2.CO.N(CH_3).CH_2.COOH$ (SCHULTZEN [1]). Likewise *taurine,* aminoethylsulphonic acid, which acts somewhat differently in different animals (SALKOWSKI [2]), passes in human beings, at least in part, into the corresponding uramino-acid, *taurocarbamic acid,* $NH_2.CO.NH.C_2H_4.SO_2.OH$. A part of the taurine also appears as such in the urine. In rabbits, when taurine is introduced into the stomach nearly all its sulphur appears in the urine as sulphuric and *hyposulphurous* acids. After subcutaneous injection the taurine appears again in great part unchanged in the urine. In dogs a great part of the sulphur of *cystine* appears in the urine as sulphate (also as thiosulphate) (BLUM, ABDERHALDEN and SAMUELY [3]).

The *nitriles,* including hydrocyanic acid, pass, according to LANG, into sulphocyanide combinations, and this sulphocyanide apparently originates from the non-oxidized sulphur of the proteins, which is readily split off. PASCHELES' observations indicate that, in an alkaline reaction and at the temperature of the body, this sulphur can convert the alkali-cyanides readily into sulphocyanides. The alkali sulphocyanides when ingested are nearly quantitatively eliminated in the urine, according to POLLAK.[4]

By *substitution with halogens,* bodies otherwise readily oxidizable are converted into difficultly oxidizable ones. While the aldehydes are readily and completely burnt like the primary and secondary alcohols of the fatty series, the halogen substituted aldehydes and alcohols are, on the contrary, difficultly oxidizable. The halogen substitution products of methane (chloroform, iodoform, and bromoform) are at least in part destroyed and the corresponding alkali compounds of the halogen pass into the urine.[5]

By *conjugation with sulphuric acid,* the alcohols which are otherwise readily oxidizable may be guarded against combustion, and consequently the alkali salt of ethylsulphuric acid is not burnt in the body (SALKOWSKI [6]).

The *organic combinations containing sulphur* act somewhat differently. W. SMITH states that the sulphur of the thio-acids, like thioglycolic acid,

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[1] Ber. d. deutsch. chem. Gesellsch., 5. See also Baumann and v. Mering, *ibid.*, 8, 584, and E. Salkowski, Zeitschr. f. physiol. Chem., 4, 107.

[2] Ber. d. deutsch. chem. Gesellsch., 6, and Virchow's Arch., 58.

[3] Blum, Hofmeister's Beiträge, 5; Abderhalden and Samuely, Zeitschr. f. physiol. Chem., 46.

[4] Lang, Arch. f. exp. Path. u. Pharm., 34; Pascheles, *ibid.*; Pollak, Hofmeister's Beiträge, 2.

[5] See Harnack and Gründler, Berlin. klin. Wochenschr., 1883; Zeller, Zeitschr. f. physiol. Chem., 8; Kast, *ibid.*, 11; Binz, Arch. f. exp. Path. u. Pharm., 28; Zeehuisen, Maly's Jahresber., 23.

[6] Pflüger's Arch., 4.

$CH_2.SH.COOH$, is in part oxidized to sulphuric acid, and according to GOLDMANN the same result occurs with aminothiolactic acid (cysteine) and the sulphur of the thio-alcohols (ethyl mercaptans). On the contrary, ethylsulphide, sulphonic and sulpho acids in general (SALKOWSKI, SMITH [1]) are not changed into sulphuric acid. Oxyethylsulphonic acid, $HO.C_2H_4.SO_2.OH$, which is in part oxidized to sulphuric acid, is an exception (SALKOWSKI).

Conjugation with glucuronic acid occurs, according to the investigations of SUNDVIK and especially of O. NEUBAUER, in many substituted as well as non-substituted alcohols, aldehydes, and ketones. *Chloral hydrate*, $C_2Cl_3OH + H_2O$, passes, after it has been converted into trichlorethylalcohol by a reduction, into a levogyrate reducing acid, *urochloralic acid* or trichlorethylglucuronic acid, $C_2Cl_3H_2.C_6H_4O_7$ (MUSCULUS and v. MERING). Of the primary alcohols investigated by NEUBAUER [2] (upon rabbits and dogs) methyl alcohol gave no conjugated glucuronic acid, and ethyl alcohol only a small amount. Isobutyl alcohol and active amyl alcohol yielded relatively large quantities. Secondary alcohols produced conjugated glucuronic acids, and indeed to a greater extent than the primary alcohols, especially in rabbits. The ketones are reduced in part into secondary alcohols and are partly excreted as the conjugated acid. This could be shown for acetone with rabbits but not with dogs.

The **homo-** and **heterocyclic compounds** pass, as far as is known, into the urine as such, or, after a previous partial oxidation or synthesis with other bodies, and appear as so-called aromatic compounds. That the benzene ring is destroyed in the body in certain cases is very probable.

The fact that benzene may be oxidized outside of the body into carbon dioxide, oxalic acid, and volatile fatty acids has been known for a long time; and as in these cases a rupture of the benzene ring must take place, so also, it must be admitted, when aromatic substances undergo a combustion in the animal body, a splitting of the benzene nucleus with the formation of fatty bodies must be the result. If this does not occur, then the benzene nucleus is eliminated with the urine as an aromatic compound of one kind or another. As the benzene nucleus can protect a substance belonging to the fatty series from destruction when conjugated with it, which is the case with the glycocoll of hippuric acid, it seems that the aromatic nucleus itself may likewise be protected from oxidation in the organism by synthesis with other bodies. The aromatic ethereal-sulphuric acids are examples of this kind.

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[1] Smith, Pflüger's Arch., **53**, **55**, **57**, and Zeitschr. f. physiol. Chem., **17**; Salkowski, Virchow's Arch., **66**; Pflüger's Arch., **39**; Goldmann, Zeitschr. f. physiol. Chem., **9**; also Baumann and Kast, *ibid.*, **14**.

[2] Sundvik, Maly's Jahresber., **16**; Musculus and v. Mering, Ber. d. deutsch. chem. Gesellsch., **8**; also v. Mering, *ibid.*, **15**; Zeitschr. f. physiol. Chem., **6**; Külz, Pflüger's Arch., **28** and **33**; O. Neubauer, Arch. f. exp. Path. u. Pharm., **46**.

The difficulty in deciding whether the benzene ring itself is destroyed in the body lies in the fact that we do not know all the different aromatic transformation products which may be produced by the introduction of any such substance into the organism, and which must be sought for in the urine. On this account it is also impossible to learn by exact quantitative determinations whether or not an aromatic substance ingested or absorbed appears again unchanged in the urine. Certain observations render it probable that the benzene ring, as above mentioned, is at least in certain cases destroyed in the body. SCHOTTEN, BAUMANN, and others have found that certain amino-acids, such as *phenylamino-propionic acid*, *amino-cinnamic acid*, and *tyrosine*, when introduced into the body cause no increase in the quantity of known aromatic substances in the urine; this makes a destruction of these amino-acids in the animal body seem probable. According to F. KNOOP[1] phenyl-α-lactic acid and phenyl-α-ketopropionic acid (phenyl pyroracemic acid) have a similar behavior, while JUVALTA's statement that phthalic acid is destroyed in the animal body is denied by E. PRIBRAM.[2] The benzene derivatives vary in behavior according to the position of the substitution, for, as found by R. COHN,[3] among the di-derivatives the ortho-compounds are more readily destroyed than the corresponding meta- or para-compounds.

An *oxidation* in the side chain of aromatic compounds is often found, and may also occur in the nucleus itself. As an example, benzene is first oxidized to oxybenzene (SCHULTZEN and NAUNYN), and this is then further in part oxidized into *dioxybenzenes* (BAUMANN and PREUSSE). *Naphthalene* appears to be converted into *oxynaphthalene*, and probably a part also into *dioxynaphthalene* (LESNIK and M. NENCKI). The hydrocarbon with an amino- or imino-group may also be oxidized by a substitution of hydroxyl for hydrogen, especially when the formation of a derivative in the para-position is possible (KLINGENBERG). For example, *aniline*, $C_6H_5.NH_2$, passes into paraminophenol, which latter passes into the urine as its ethereal-sulphuric acid, $H_2N.C_6H_4.O.SO_2.OH$ (F. MÜLLER). *Acetanilid* is in part converted into acetyl paraminophenol (JAFFÉ and

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[1] Schotten, Zeitschr. f. physiol. Chem., 7 and 8; Baumann, *ibid.*, **10**, 130. In regard to the behavior of tyrosine, see especially Blendermann, *ibid.*, **6**; Schotten, *ibid.*, **7**; Bass, *ibid.*, **11**; and R. Cohn, *ibid.*, **14**; F. Knoop, Der Abbau aromatischer Fettsäuren im Tierkörper, Habilit.-Schrift, Freiburg, 1904.

[2] Juvalta, Zeitschr. f physiol. Chem., **13**; Pribram, Arch. f. exp. Path. u. Pharm., **51**.

[3] Zeitschr. f. physiol. Chem., **17**.

HILBERT, K. MÖRNER), and *carbazol* into oxycarbazol (KLINGENBERG [1]).

An *oxidation of the side chain* may occur by the hydrogen atoms being replaced by hydroxyl, as in the oxidation of *indol* and *skatol* into indoxyl and skatoxyl. An oxidation of the side chain may also take place with the formation of carboxyl; thus, for example, *toluene*, $C_6H_5.CH_3$ (SCHULTZEN and NAUNYN), *ethyl-benzene*, $C_6H_5.C_2H_5$, and *propylbenzene*, $C_6H_5.C_3H_7$ (NENCKI and GIACOSA [2]), besides many other bodies, are oxidized into benzoic acid. *Cymene* is oxidized to cumic acid, *xylene* to toluic acid, *methylpyridine* to pyridine-carboxylic acid in the same way. If the side chain has several members, the behavior is somewhat different. *Phenylacetic acid*, $C_6H_5.CH_2.COOH$, in which only one carbon atom exists between the benzene nucleus and the carboxyl, is not oxidized, but is eliminated after conjugation with glycocoll as *phenaceturic acid* (SALKOWSKI [3]). *Phenylaminoacetic acid*, $C_6H_5.CHNH_2.COOH$ is in part converted into *mandelic acid* (phenylglycollic acid), $C_6H_5.CHOH.COOH$, and in great part is eliminated as such (SCHOTTEN, KNOOP [4]). *Phenylpropionic acid*, $C_6H_5.CH_2.CH_2.COOH$, with three carbon atoms in the side chain, is, on the contrary, oxidized into benzoic acid, and H. and E. SALKOWSKI [5] have proposed the rule that the homologues of the benzoic acids are converted into benzoic acid when the side chain contains more than two carbon atoms.

KNOOP has shown by experiments with several acids, such as phenylbutyric acid, phenyl-α-lactic acid and others that this rule does not hold good. The *phenylbutyric acid*, $C_6H_5CH_2.CH_2.CH_2.COOH$, is not oxidized in the animal body into benzoic acid, but into phenylacetic acid, and the *phenyl-α-lactic acid*, $C_6H_5.CH_2.CH(OH).COOH$, is decomposed nearly entirely and only a small residue is eliminated unchanged. KNOOP has, on the contrary, made it very probable that, at least for the saturated, normal fatty acids with phenyl substituted at the end, on their oxidation they follow the rule that the carboxyl group produced from the body stands in the β position to the original carboxyl. This explains, for example, the formation of phenylacetic acid from phenylbutyric acid, and benzoic acid from phenylvalerianic acid, $C_6H_5.CH_2.CH_2.CH_2.CH_2.COOH$, for in the last-mentioned case phenylpropionic acid must first be produced and then benzoic acid from this. Exceptions to this rule are the propionic acids substituted in the α-position, i.e., phenylalanine, phenyl-α-lactic acid, and phenyl-α-ketopropionic acid, which, like tyrosine and α-amino-cinnamic acid, are burnt in the body. SCHOTTEN's rule, according to which all acids having three carbon atoms in the side chain of which the middle one has a NH_2 group attached, are nearly completely burnt in the organism, has been extended by these exceptions.

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[1] Schultzen and Naunyn, Reichert and Arch. f. (Anat. u.) Physiol., 1867; Baumann and Preusse, Zeitschr. f. physiol. Chem., **3**, 156. See also Nencki and Giacosa, *ibid.*, **4**; Lesnik and Nencki, Arch. f. exp. Path. u. Pharm., **24**; F. Müller, Deutsch. med. Wochenschr., 1887; Jaffé and Hilbert, Zeitschr. f. physiol. Chem., **12**; Mörner, *ibid.*, **13**; Klingenberg, "Studien über die Oxydation aromatischer Substanzen," etc. Inaug.-Diss. Rostock, 1891. In regard to formanilid, which acts essentially as acetanilid, see Kleine, Zeitschr. f. physiol. Chem., **22**.

[2] Zeitschr. f. physiol. Chem., **4**.

[3] *Ibid.*, **7** and **9**.

[4] *Ibid.*, **8**.

[5] *Ibid.*, **7**.

If *several side chains* are present in the benzene nucleus, then only one is always oxidized into carboxyl. Thus *xylene*, $C_6H_4(CH_3)_2$, is oxidized into *toluic acid*, $C_6H_4(CH_3)COOH$ (SCHULTZEN and NAUNYN); *mesitylene*, $C_6H_3(CH_3)_3$. into *mesitylenic acid*, $C_6H_3(CH_3)_2.COOH$ (L. NENCKI); *cymene*, $(CH_3)_2CH.C_6H_4.CH_3$, into *cumic acid* (M. NENCKI and ZIEGLER[1]); and *vanillin*, $OH.C_6H_3<\substack{OCH_3\\CHO}$, into, *vanillinic acid* (Y. KOTAKE).[2]

Reductions may also occur and examples of this kind are the conversion, as observed by E. MEYER,[3] of *nitrobenzene*, $C_6H_5NO_2$, or of *nitrophenol*, $HO.C_6H_4.NO_2$ into aminophenol, $HO.C_6H_4.NH_2$, and also the behavior of *m*-nitrobenzaldehyde in the animal body as mentioned below.

Syntheses of aromatic substances with other atomic groups occur frequently. To these syntheses belongs, in the first place, the conjugation of *benzoic acid* with *glycocoll* to form *hippuric acid*, the discovery of which is generally ascribed to WÖHLER, but according to HEFFTER[4] more correctly to KELLER and URE. All the numerous aromatic substances which are converted into benzoic acid in the body are voided partly as hippuric acid. This statement is not true for all species of animals. According to the observations of JAFFÉ,[5] benzoic acid does not pass into hippuric acid in birds, but into another nitrogenous acid, *ornithuric acid*, $C_{19}H_{20}N_2O_4$. This acid yields as splitting products, besides benzoic acid, *ornithine*, a body which has been spoken of on page 97. Not only are the *oxybenzoic acids* and the *substituted benzoic acids* conjugated with glycocoll, forming corresponding hippuric acids, but also the above-mentioned acids, *toluic*, *mesitylenic*, *cumic*, and *phenylacetic acids*. These acids are voided as *toluric*, *mesitylenuric*, *cuminuric*, and *phenaceturic acids*.

It must be remarked in regard to the oxybenzoic acids that a conjugation with glycocoll has been shown only with salicylic and *p*-oxybenzoic acid (BERTAGNINI, BAUMANN, HERTER, and others), while BAUMANN and HERTER[1] find it only very probable for *m*-oxybenzoic acid. The oxybenzoic acids are also in part eliminated as conjugated sulphuric acids, which is especially true for *m*-oxybenzoic acid. The three aminobenzoic acids, according to the experiments of HILDEBRANDT, on rabbits, appeared at least in part unchanged in the urine. SALKOWSKI found, as was later confirmed by R. Cohn,[2] that *m*-aminobenzoic acid passes in part into *uraminobenzoic acid*, $H_2N.CO.HN.C_6H_4.COOH$. It is also in part eliminated as aminohippuric acid.

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[1] L. Nencki, Arch. f. exp. Path. u. Pharm., **1**; Nencki and Ziegler, Ber. d. deutsch. chem. Gesellsch., **5**. See also O. Jacobsen, *ibid.*, **12**.

[2] Zeitschr. f. physiol. Chem., **45**.

[3] *Ibid.*, **46**.

[4] Die Ausscheidung körperfremder Substanzen im Harn. Ergebnisse der Physiol., **4**, 252.

[5] Ber. d. d. chem. Gesellsch., **10** and **11**.

The behavior of the halogen-substituted compounds of toluene varies in different animals according to HILDEBRANDT's experiments. In dogs they are converted into the corresponding substituted hippuric acid. In rabbits *o*-bromtoluene is completely changed to hippuric acid, the *m*- and *p*-bromtoluene only partly. The three chlortoluenes are converted in rabbits into the corresponding benzoic acid and are eliminated as such and not as hippuric acid.

The substituted aldehydes are of special interest as substances which may undergo conjugation with glycocoll. According to the investigations of R. COHN[3] on this subject *o-nitrobenzaldehyde* when introduced into a rabbit is only in a very small part converted into nitrobenzoic acid, and the chief mass, about 90 per cent, is destroyed in the body. According to SIEBER and SMIRNOW[4] *m-nitrobenzaldehyde* passes in dogs into *m*-nitrohippuric acid, and according to COHN into urea-*m*-nitrohippurate. In rabbits the behavior is quite different. In this case not only does an oxidation of the aldehyde into benzoic acid take place, but the nitro-group is also reduced to an amino-group, and finally acetic acid attaches itself to this with the expulsion of water, so that the final product is *m-acetylaminobenzoic acid*, $CH_3.CO.NH.C_6H_4.COOH$. This process is analogous to the behavior of furfurol, and the reduction does not take place in the intestine, but in the tissues. The *p*-nitrobenzaldehyde acts in rabbits in part like the *m*-aldehyde and passes in part into *p-acetylaminobenzoic acid*. Another part is converted into *p*-nitrobenzoic acid, and the urine contains a chemical combination of equal parts of these two acids. According to SIEBER and SMIRNOW *p*-nitrobenzaldehyde yields only urea *p*-nitrohippurate in dogs. The above-mentioned *pyridine-carboxylic acid*, formed from methylpyridine (α-picoline) passes into the urine after conjugation with glycocoll as *α-pyridinuric acid.*[1]

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[1] Zeitschr. f. physiol. Chem., **1**, where Bertagnini's work is also cited. See also Dautzenberg, Maly's Jahresber., **11**, 231.

[2] Salkowski, Zeitschr. f. physiol. Chem., **7**; Cohn, *ibid.*, **17**; Hildebrandt, Hofmeister's Beiträge, **3**.

[3] Zeitschr. f. physiol. Chem., **17**.

[4] Monatshefte f. Chem., **8**.

To those substances which undergo a conjugation with glycocoll belongs also *furfurol* (the aldehyde of pyromucic acid), which, when introduced into rabbits and dogs, as shown by JAFFÉ and COHN,[2] is first oxidized into pyromucic acid and then eliminated as *pyromucuric acid*. $C_7H_7N_4O$, after conjugation with glycocoll. In birds this behavior is different, namely, the acid is conjugated with another substance, *ornithine*, $C_5H_{12}N_2O_2$, which is a diaminovalerianic acid, forming *pyromucinornithuric acid.*

Furfurol also undergoes conjugation with glycocoll in other forms in mammals. Thus JAFFÉ and COHN found that it is in part combined with acetic acid, forming *furfuracrylic acid*, $C_4H_3O.CH:CH.COOH$, which passes into the urine coupled with glycocoll as *furfuracryluric acid.*

It has not been proved how *thiophene*, C_4H_4S, behaves in the animal body. Of *methylthiophene* (thiotolene), $C_4H_3S.CH_3$, a very small part is oxidized to thiophenic acid, $C_4H_3S.COOH$ (LEVY). This acid, as shown by JAFFÉ and LEVY,[3] is conjugated with glycocoll in the body (rabbits) and eliminated as *thiophenuric acid.*

Another very important synthesis of aromatic substances is that of the *ethereal-sulphuric acids*. *Phenols* and chiefly the *hydroxylated aromatic hydrocarbons* and their derivatives are voided as ethereal-sulphuric acids, according to BAUMANN, HERTER and others.[4]

A conjugation of aromatic acids with sulphuric acid occurs less often. The two previously-mentioned aromatic acids, *p-oxyphenylacetic* and *p-oxyphenylpropionic acid*, are in part eliminated in this form. *Gentisic acid* (hydroquinone-carboxylic acid) also increases, according to LIKHATSCHEFF,[5] the quantity of ethereal-sulphuric acid in the urine, and according to ROST the same occurs, contrary to the older statements, with *gallic acid* (trioxybenzoic acid) and *tannic acid.*[6]

While *acetophenone* (phenylmethylketone), $C_6H_5.CO.CH_3$, as shown by M. NENCKI, is oxidized to benzoic acid and eliminated as hippuric acid, the aromatic oxyketones with hydroxyl groups, such as *resacetophenone*,

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[1] In regard to the extensive literature on glycocoll conjugations we refer the reader to O. Kühling, Ueber Stoffwechselprodukte aromatischer Körper. Inaug.-Diss., Berlin, 1887.

[2] Ber. d. d. Chem. Gesellsch., **20** and **21**.

[3] Levy, Ueber das Verhalten einiger Thiophenderivate, etc., Inaug.-Diss., Königsberg, 1889; Jaffé and Levy, Ber. d. d. chem. Gesellsch., **21**.

[4] In regard to the literature, see O. Kühling, l. c.

[5] Zeitschr. f. physiol. Chem., **21**.

[6] In regard to the behavior of gallic and tannic acids in the animal body, see C. Mörner, Zeitschr. f. physiol. Chem., **16**, which also contains the older literature; also Harnack, *ibid.*, **24**, and Rost, Arch. f. exp. Path. u. Pharm., **38**, and Sitzungsber. d. Gesellsch. zur Beförd. d. ges. Naturwiss. zu Marburg, 1898.

$C_6H_3(\overset{1}{O}H)(\overset{3}{O}H)(CO.\overset{4}{C}H_3)$, *paraoxypropiophenone*, $C_6H_4(\overset{1}{O}H)(CO\overset{4}{C}H_2.CH_3)$, and *gallacetophenone*, $C_6H_2(\overset{1}{O}H)(\overset{2}{O}H)(\overset{3}{O}H)(CO.\overset{4}{C}H_3)$, pass into the urine without previous oxidation as ethereal-sulphuric acids and in part after conjugation with glucuronic acid (Nencki and Rekowski[1]). *Euxanthon*, which is also an aromatic oxyketone, passes into the urine as *euxanthic acid* after the conjugation with glucuronic acid previously mentioned.

A *conjugation* of other aromatic substances *with glucuronic acid*, which last is protected from combustion, occurs rather often. The phenols, as above stated (page 590), pass in part as conjugated glucuronic acids into the urine. The same is true for the homologues of the phenols, for certain substituted phenols, and for many aromatic substances, also hydrocarbons after previous oxidation and hydration. Thus Hildebrandt and Fromm and Clemens[2] have shown that the *cyclic terpenes* and *camphors*, by oxidation or hydration, or in certain cases by both, are converted into hydroxyl derivatives when the body in question is not previously hydroxylized, and that these hydroxyl derivatives are eliminated as conjugated glucuronic acids. Conjugated glucuronic acids are detected in the urine after the introduction of various substances, e.g., therapeutic agents into the organism, namely, *terpenes, borneol, menthol, camphor* (camphoglucuronic acid was first observed by Schmiedeberg), *naphthalene*, *oil of turpentine, oxyquinolines, antipyrine*, and many other bodies.[3] *Orthonitrotoluene* in dogs passes first into *o*-nitrobenzyl alcohol and then into a conjugated glucuronic acid, *uronitrotoluolic acid* (Jaffé).[4] The glucuronic acid split off from this conjugated acid is levogyrate and hence is not identical but only isomeric with the ordinary glucuronic acid. *Dimethylaminobenzaldehyde*, according to Jaffé, is converted in part into *dimethylaminobenzoglucuronic acid* in rabbits. The same conjugated glucuronic acid is also produced, according to Hildebrandt,[5] from *p-dimethyltoluidine*, which is first changed into *p-dimethylaminobenzoic acid*. *Indol* and *skatol* seem, as above stated (page 595), to be eliminated in the urine partly as conjugated glucuronic acids.

A synthesis in which compounds containing sulphur, *mercapturic acids*, are formed and eliminated after conjugation with glucuronic acid, occurs when chlorine and bromine derivatives of benzene are introduced into the organism of dogs (BAUMANN and PREUSSE, JAFFÉ). Thus *chlorbenzene* combines with *cysteine*, forming *chlorphenylmercapturic acid*, $C_{11}H_{12}ClSNO_3$. The important investigations of FRIEDMANN[1] show that the phenylthiolactic acid which forms the foundation of the mercapturic acids belongs to the β-series, and in this way the direct chemical connection of this body with the protein-cystine (α-amino-β-thiolactic acid) is established. FRIEDMANN has also been able to convert cysteine into bromphenylmercapturic acid.

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[1] Arch. d. scienc. biol. de St. Pétersbourg, **3**, and Ber. d. deutsch. chem. Gesellsch., **27**.

[2] Hildebrandt, Arch. f. exp. Path. u. Pharm., **45**, **46**; Zeitschr. f. physiol. Chem., **36**; with Fromm, *ibid.*, **33**; and with Clemens, *ibid.*, **37**; Fromm and Clemens, *ibid.*, **34**.

[3] See O. Kühling, l. c., which gives the literature up to 1887; also E. Külz, Zeitschr. f. Biologie, **27**; the works of Hildebrandt, Fromm and Clemens, see foot-note, 2; Brahm, Zeitschr. f. physiol. Chem., **28**; Fenyvessy, *ibid.*, **30**; Bonanni, Hofmeister's Beiträge, **1**; Lawrow, Ber. d. d. chem. Gesellsch., **33**.

[4] Zeitschr. f. physiol. Chem., **2**.

[5] Jaffé, Zeitschr. f. physiol. Chem., **43**; Hildebrandt, Hofmeister's Beiträge, **7**.

Pyridine, C_5H_5N, which does not combine either with glucuronic acid or with sulphuric acid after previous oxidation, shows a special behavior. It takes up a methyl group as found by HIS and later confirmed by COHN,[2] and forms an ammonium combination, *methylpyridylammonium hydroxide*, $HO.CH_3.NC_5H_5$.

Several alkaloids, such as *quinine*, *morphine*, and *strychnine*, may pass into the urine. After the ingestion of *turpentine*, *balsam of copaiva*, and *resins*, these may appear in the urine as resin acids. Different kinds of coloring-matters, such as *alizarin*, *crysophanic acid*, after rhubarb or senna, and the *coloring-matter of the blueberry*, etc., may also pass into the urine. After *rhubarb*, *senna*, or *santonine* thc urine assumes a yellow or greenish-yellow color, which is transformed into a beautiful red by the addition of alkali. *Phenol* produces, as above mentioned, a dark-brown or dark-green color which depends mainly on the decomposition products of hydroquinone and humin substances. After *naphthalene* the urine has a dark color, and several other medicinal agents produce a special coloration. Thus after antipyrine it becomes yellow or blood-red. After *balsam of copaiva* the urine becomes, when strongly acidified with hydrochloric acid, gradually rose- and purple-red. After *naphthalene* or *naphthol* the urine gives with concentrated sulphuric acid (1 c.c. of concentrated acid and a few drops of urine) a beautiful emerald-green color, which is probably due to naphthol-glucuronic acid. Odoriferous bodies also pass into the urine. After asparagus the urine acquires a disgusting odor which is probably due to methylmercaptan, according to M. NENCKI.[3] After turpentine the urine may have a peculiar odor similar to that of violets.

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[1] Baumann and Preusse, Zeitschr. f. physiol. Chem., 5; Jaffé, Ber. d. deutsch. chem. Gesellsch., 12; Friedmann, Hofmeister's Beiträge, 4.

[2] His, Arch. f. exp. Path. u. Pharm., **22**; Cohn, Zeitschr. f. physiol. Chem., **18**.

[3] Arch. f. exp. Path. u. Pharm., **28**.

VI. Pathological Constituents of Urine.

Proteid. The appearance of slight traces of proteid in normal urines has been repeatedly observed by many investigators, such as POSNER, PLÓSZ, v. NOORDEN, LEUBE, and others. According to K. MÖRNER [1] proteid regularly occurs as a normal urinary constituent to the extent of 22–78 milligrams per liter. Frequently traces of a substance similar to a nucleoalbumin, which is easily mistaken for mucin, and whose nature will be treated of later, appear in the urine. In diseased conditions proteid occurs in the urine in a variety of cases. The albuminous bodies which most often occur are serglobulin and seralbumin. Proteoses (or peptones) are also sometimes present. The quantity of proteid in the urine is in most cases less than 5 p. m., rarely 10 p. m., and only very rarely does it amount to 50 p. m. or over. Cases are known, however, where it was even more than 80. p m.

Among the many reactions proposed for the detection of proteid in urine, the following are to be recommended:

The Heat Test. Filter the urine and test its reaction. An acid urine may, as a rule, be boiled without further treatment, and only in especially acid urines is it necessary to first treat with a little alkali. An alkaline urine is made neutral or faintly acid before heating. If the urine is poor in salts, add 1-10 vol. of a saturated common-salt solution before boiling; then heat to the boiling-point, and if no precipitation, cloudiness, or opalescence appears, the urine in question contains no coagulable proteid, but it may contain proteoses or peptones. If a precipitate is produced on boiling, this may consist of proteid, or of earthy phosphates, or of both. The monohydrogen calcium phosphate decomposes on boiling, and the normal phosphate may separate out. The proper amount of acid is now added to the urine, so as to prevent any mistake caused by the presence of earthy phosphates, and to give a better and more flocculent precipitate of the proteid. If acetic acid is used for this, then add 1–3 drops of a 25 per cent acid to each 10 c.c. of the urine and boil after the addition of each drop. On using nitric acid, add 1–2 drops of the 25 per cent acid to each cubic centimeter of the boiling-hot urine.

On using acetic acid, when the quantity of proteid is very small, and especially when the urine was originally alkaline, the proteid may sometimes remain in solution on the addition of the above quantity of acid. If, on the contrary, less acid is added, the precipitate of calcium phosphate, which forms in amphoteric or faintly acid urines, is liable not to dissolve completely, and this may cause it to be mistaken for a proteid precipitate. If nitric acid is used for the heat test, the fact must not be overlooked that after the addition of only a little acid a combination between it and the proteid is formed which is soluble on boiling and which is only precipitated by an excess of the acid. On this account the large quantity of nitric acid, as suggested above, must be added, but in this case a small part of the proteid is liable to be dissolved by the excess of the nitric acid. When the acid is added after boiling, which is absolutely necessary, the liability of a mistake is not so great. It is on these grounds that the heat test, although it gives very good results in the hands of experts, is not recommended to physicians as a positive test for proteid.

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[1] Skand. Arch. f. Physiol., 6 (literature).

A confounding with mucin, when this body occurs in the urine, is easily prevented in the heat test with acetic acid by acidifying another portion with acetic acid at the ordinary temperature. Mucin and nucleoalbumin substances similar to mucin are hereby precipitated. If in the performance of the heat and nitric-acid test a precipitate first appears on cooling or is strikingly increased, then this shows the presence of proteoses in the urine, either alone or mixed with coagulable proteid. In this case a further investigation is necessary (see below). In a urine rich in urates a precipitate consisting of uric acid separates on cooling. This precipitate is colored and granular, and is hardly to be mistaken for an proteoses or proteid precipitate.

HELLER'S *test* is performed as follows (see page 41): The urine is very carefully floated on the surface of nitric acid in a test-tube. The presence of proteid is shown by a white ring between the two liquids. With this test a red or reddish-violet transparent ring is always obtained with normal urine; it depends upon the indigo coloring-matters and can hardly be mistaken for the white or whitish proteid ring, and this last must not be mistaken for the ring produced by bile-pigments. In a urine rich in urates another complication may occur, due to the formation of a ring produced by the precipitation of uric acid. The uric-acid ring does not lie, like the proteid ring, between the two liquids, but somewhat higher. For this reason two simultaneous rings may exist in urines which are rich in urates and do not contain very much proteid. The disturbance caused by uric acid is easily prevented by diluting the urine with 1–2 vols. of water before performing the test. The uric acid now remains in solution, and the delicacy of HELLER'S test is so great that after dilution only in the presence of insignificant traces of proteid does this test give negative results. In a urine very rich in urea a ring-like separation of urea nitrate may also appear. This ring consists of shining crystals, and it does not appear in urine previously diluted. A confusion with resinous acids, which also give a whitish ring with this test, is easily prevented, since these acids are soluble on the addition of ether. Stir, add ether, and carefully shake the contents of the test-tube. If the cloudiness is due to resinous acids, the urine gradually becomes clear, and on evaporating the ether a sticky residue of resinous acids is obtained. A liquid which contains true mucin does not give a precipitate with this test, but it gives a more or less strongly opalescent ring, which disappears on stirring. The liquid does not contain any precipitate after stirring, but is somewhat opalescent. If a faint, not wholly typical reaction is obtained with HELLER'S test after some time with undiluted urine, while the diluted urine gives a pronounced reaction, the presence is shown of the substance which used to be called mucin or nucleoalbumin. In this case proceed as described below for the detection of nucleoalbumin.

If the above-mentioned possible errors and the means by which they may be prevented are borne in mind, there is hardly another test for proteid in the urine which is at the same time so easily performed, so delicate, and so positive as HELLER'S. With this test even 0.002 per cent of albumin may be detected without difficulty. Still the student must not be satisfied with this test alone, but should apply at least a second one, such as the heat test. In performing this test the (primary) proteoses are also precipitated.

The *reaction with metaphosphoric acid* (see page 41) is very convenient and easily performed. It is not quite so delicate and positive as HELLER's test. The proteoses are also precipitated by this reagent.

Reaction with Acetic Acid and Potassium Ferrocyanide. Treat the urine first with acetic acid until it contains about 2 per cent, and then add drop by drop a potassium-ferrocyanide solution (1:20), carefully avoiding an excess. This test is very good, and in the hands of experts it is even more delicate than HELLER's. In the presence of very small quantities of proteid it requires more practice and dexterity than HELLER's, as the relative quantities of reagent, proteid, and acetic acid influence the result of the test. The quantity of salts in the urine likewise seems to have an influence. This reagent also precipitates proteoses.

SPIEGLER'S *Test.* SPIEGLER recommends a solution of 8 parts mercuric chloride, 4 parts tartaric acid, 20 parts glycerine, and 200 parts water as a very delicate reagent for proteid in the urine. A test-tube is half filled with this reagent and from a pipette the urine is allowed to flow upon its surface drop by drop along the wall of the test-tube. In the presence of proteid a white ring is obtained at the point of contact between the two liquids. The delicacy of this test is 1:350;000. JOLLES [1] does not consider this reagent suited for urines very poor in chlorine, and for this reason he has changed it as follows: 10 grams mercuric chloride, 20 grams succinic acid, 10 grams NaCl, and 500 c.c. water.

ROCH'S *Test.* Treat the urine either with a 20 per cent watery solution of sulphosalicylic acid or a few crystals of the acid. This reagent does not precipitate the uric acid or the resin acids.[2]

As every normal urine contains traces of proteid, it is apparent that very delicate reagents are to be used only with the greatest caution. For ordinary cases HELLER's test is sufficiently delicate. If no reaction is obtained with this test within 2½ to 3 minutes, the urine tested contains less than 0.003 per cent of proteid, and is to be considered free from proteid in the ordinary sense.

The use of precipitating reagents presumes that the urine to be investigated is perfectly clear, especially in the presence of only very little proteid. The urine must first be filtered. This is not easily done with urine containing bacteria, but a clear urine may be obtained, as suggested by A. JOLLES, by shaking the urine with infusorial earth. Although a little proteid is retained in this procedure and lost, it does not seem to be of any importance (GRÜTZNER, SCHWEISSINGER [3]).

The different *color reactions* cannot be directly used, especially in deep-colored urines which contain only little proteid. The common salt of the urine has a disturbing action on MILLON's reagent. To prove more positively the presence of proteid, the precipitate obtained in the boiling test may be filtered, washed, and then tested with MILLON's reagent. The precipitate may also be dissolved in dilute alkali and the biuret test applied to the solution. The presence of proteoses or peptones in the urine is directly tested for by this last-mentioned test. In testing the urine for proteid one should never be satisfied with one reaction alone, but must apply the heat test and HELLER's or the potassium-ferrocyanide test. In using the heat test alone the proteoses may be easily overlooked, but these are detected, on the contrary, by HELLER's or the potassium-ferrocyanide test. If only one of these tests is employed, no sufficient intimation of the kind of proteid present can be obtained, whether it consists of proteoses or coagulable proteid.

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[1] Spiegler, Wien. klin. Wochenschr., 1892, and Centralbl. f. d. klin. Med., 1893; Jolles, Zeitschr. f. physiol. Chem., **21**.

[2] Pharmaceut. Centralbl., 1889, and Zeitschr. f. physiol. Chem., **29**.

[3] Jolles, Zeitschr. f. anal. Chem., **29**; Grützner, Chem. Cenrtalbl., 1901, **1**; Schweissinger, *ibid.*

For practical purposes several dry reagents for proteid have been recommended. Besides the metaphosphoric acid may be mentioned STUTZ's or FÜRBRINGER's gelatine capsules, which contain mercuric chloride, sodium chloride, and citric acid; and GEISSLER's albumin-test papers, which consist of strips of filter-paper which have been dipped in a solution of citric acid and also mercuric-chloride and potassium-iodide solution and then dried.

If the presence of proteid has been positively proved in the urine by the above tests, it then remains necessary to determine its character.

The *Detection of Globulin and Albumin.* In detecting serglobulin the urine is exactly neutralized, filtered, and treated with magnesium sulphate in substance until it is completely saturated at the ordinary temperature, or with an equal volume of a saturated neutral solution of ammonium sulphate. In both cases a white, flocculent precipitate is formed in the presence of glubulin. In using ammonium sulphate with a urine rich in urates a precipitate consisting of ammonium urate may separate. This precipitate does not appear immediately, but only after a certain time, and it must not be mistaken for the globulin precipitate. In detecting seralbumin heat the filtrate from the globulin precipitate to boiling-point, or add about 1 per cent acetic acid to it at the ordinary temperature.

For the detection and also for the quantitative estimation of the various globulins (fibringlobulin, euglobulin, and pseudoglobulin) OSWALD[1] has proposed the fractional precipitation with ammonium sulphate. It is still a question whether this method, which is not quite reliable, can be used in urine investigations.

Proteoses and *peptones* have been repeatedly found in the urine in different diseases. Reliable reports are at hand on the occurrence of proteoses in the urine. The statements in regard to the occurrence of peptones date from a time when the conception of proteoses and peptones was different from that of the present day, and in part they are based upon investigations using untrustworthy methods. According to ITO[2] true peptones are sometimes found in the urine in cases of pneumonia; what has been designated as urine peptone seems to have been chiefly deuteropeptones.

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[1] Münch. med. Wochenschr., 1904.

[2] In regard to the literature on proteoses and peptones in urine, see Huppert-Neubauer, Harn-Analyse, 10. Aufl., 466 to 492; also A. Stoffregen, Ueber das Vorkommen von Pepton im Harn, Sputum und Eiter (Inaug.-Diss., Dorpat, 1891); E. Hirschfeldt, Ein Beitrag zur Frage der Peptonurie (Inaug.-Diss., Dorpat, 1892); and especially Stadelmann, Untersuchungen über die Peptonurie. Wiesbaden, 1894; Ehrström, Bidrag till kännedomen om Albumosurien, Helsingfors, 1900; Ito, Deutsch. Arch. f. klin. Med., 71.

In detecting the proteoses the proteid-free urine, or urine boiled with addition of acetic acid, is saturated with ammonium sulphate, which precipitates the proteoses. Several errors are here possible. The urobilin, which may give a reaction similar to the biuret reaction, is also precipitated and may lead to mistakes (SALKOWSKI, STOKVIS[1]). A small quantity of the proteid may remain in solution after coagulation and this may be precipitated by the ammonium sulphate and be mistaken for proteoses. The coagulable proteid may be completely precipitated by saturating with ammonium sulphate in boiling solution; but according to DEVOTO[2] small quantities of proteose may be formed from the proteid by heating for a long time with the salt. On heating for a short time no such formation of proteose takes place, and the proteids are completely coagulated.

For these reasons BANG[3] has suggested the following method for the detection of proteoses in the presence of coagulable proteid. The urine is heated to boiling with ammonium sulphate (8 parts to 10 parts urine) and boiled for a few seconds. The hot liquid is centrifuged for ½ to 1 minute and separated from the sediment The urobilin is removed from this by extraction with alcohol. The residue is suspended in a little water, heated to boiling, filtered, whereby the coagulable proteid is retained on the filter, and any urobilin still present in the filtrate is shaken out with chloroform. The watery solution, after removal of the chloroform, is used for the biuret test. For clinical purposes this method is very serviceable.

According to SALKOWSKI the urine treated with 10 per cent hydrochloric acid is precipitated with phosphotungstic acid, then warmed, the liquid decanted from the resin-like precipitate, this washed with water, and then dissolved in a little water with the aid of some caustic soda, warmed again until the blue color disappears, cooled, and finally tested with copper sulphate. This method has been recently somewhat modified by v. ALDOR and ČERNY.[4] In regard to other more complicated methods we refer to HUPPERT-NEUBAUER.

MORAWITZ and DIETSCHY[5] first remove the proteid from the urine made faintly acid with acid potassium phosphate by the addition of double the volume of 96 per cent alcohol and warming on the water-bath for several hours. From the concentrated filtrate acidified with a little sulphuric acid the proteoses can be precipitated by saturating with zinc sulphate. After the removal of the urobilin by alcohol and extracting with water, the biuret test may be applied.

If the proteoses have been precipitated from a larger portion of urine by ammonium sulphate, this precipitate is tested for the presence of different proteoses for the reasons given in Chapter II. The following serves as a preliminary determination of the character of the proteoses present in the urine. If the urine contains only deuteroproteose it does not become cloudy on boiling, does not give HELLER's test, does not become cloudy on saturating with NaCl in neutral reaction, but does become turbid on adding acetic acid saturated with this salt. In the presence of only protoproteose the urine gives HELLER's test, is precipitated even in neutral solution on saturating with NaCl, but does not coagulate on boiling. The presence of heteroproteose is shown by the urine behaving like the above with NaCl and nitric acid, but shows a difference on heating. It gradually becomes cloudy on warming and separates at about 60° C. a sticky precipitate which attaches itself to the sides of the vessel and which dissolves at boiling temperature on acidifying the urine; the precipitate reappears on cooling.

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[1] Salkowski, Berlin. klin. Wochenschr., 1897; Stokvis, Zeitschr. f. Biologie, 34.

[2] Zeitschr f. physiol. Chem , 15.

[3] Deutsch med. Wochenschr., 1898.

[4] Salkowski, Centralbl. f. d. med. Wissensch., 1894; v. Aldor, Berl. klin. Wochenschr., 36; Černy Zeitschr. f. analyt. Chem., 40.

[5] Arch. f. exp Path. u. Pharm., 54.

In close relation to the proteoses stands the so-called BENCE-JONES proteid, which occurs in the urine in rare cases in disease with changes in the spinal marrow. It gives a precipitate on heating to 40–60° C., which on further heating to boiling dissolves again more or less completely, depending upon the reaction and upon the amount of salt present. It does not separate on dialysis, but can be precipitated from the urine by double the volume of a saturated ammonium-sulphate solution or by alcohol. It has also been obtained as crystals (GRUTTERINK and DE GRAAFF, MAGNUS-LEVY [1]). This body shows a somewhat different behavior in the various cases in which it has been found and its nature has not been explained. From the investigations of the above-mentioned and other experimenters (MOITESSIER, ABDERHALDEN and ROSTOSKI) we can draw the conclusion that this proteid is similar to the proteoses in several reactions, but that nevertheless it stands close to the genuine protein bodies. It also yields primary as well as secondary proteoses on peptic digestion (GRUTTERINK and DE GRAAFF), and yields the same hydrolytic cleavage products as the other proteids (ABDERHALDEN and ROSTOSKI).

Quantitative Estimation of Proteid in Urine. Of all the methods proposed thus far, the COAGULATION METHOD (boiling with the addition of acetic acid) when performed with sufficient care gives the best results. The average error need never amount to more than 0.01 per cent, and it is generally smaller. With this method it is best to first find how much acetic acid must be added to a small portion of the urine, which has been previously heated on the water-bath, to completely separate the proteid so that the filtrate will not respond to HELLER's test. Then coagulate 20–50–100 c.c. of the urine. Pour the urine into a beaker and heat on the water-bath, add the required quantity of acetic acid slowly, stirring constantly, and heat at the same time. Filter while warm, wash first with water, then with alcohol and ether, dry and weigh, incinerate and weigh again. In exact determinations the filtrate must not give HELLER's test.

The separate estimation of GLOBULINS and ALBUMINS is done by carefully neutralizing the urine and precipitating with $MgSO_4$ added to saturation (HAMMARSTEN), or simply by adding an equal volume of a saturated neutral solution of ammonium sulphate (HOFMEISTER and POHL [1]). The precipitate consisting of globulin is thoroughly washed with a saturated magnesium-sulphate or half-saturated ammonium-sulphate solution, dried continuously at 110° C., boiled with water, extracted with alcohol and ether, then dried, weighed, incinerated, and weighed again. The quantity of albumin is calculated as the difference between the quantity of globulin and the total proteids.

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[1] Magnus-Levy, Zeitschr. f. physiol. Chem., **30** (literature); Grutterink and de Graaff, *ibid.*, **34** and **46**; Moitessier, Compt. rend. soc. biolog., **57**; Abderhalden and Rostoski, Zeitschr. f. physiol. Chem., **46**.

Approximate Estimation of Proteid in Urine. Of the methods suggested for this purpose none has been more extensively employed than ESBACH'S.

ESBACH'S [2] *Method.* The acidified urine (with acetic acid) is poured into a specially graduated tube to a certain mark, and then the reagent (a 2 per cent citric-acid and 1 per cent picric-acid solution in water) is added to a second mark, the tube closed with a rubber stopper and carefully shaken, avoiding the production of froth. The tube is allowed to stand twenty-four hours, and then the height of the precipitate on the graduation is read off. The reading gives directly the quantity of proteid in 1000 parts of the urine. Urines rich in proteid must first be diluted with water. The results obtained by this method are, however, dependent upon the temperature; and a difference in temperature of 5° to 6.5° C. may cause an error of 0.2–0.3 per cent deficiency or excess in urines containing a medium quantity of proteid (CHRISTENSEN and MYGGE [3]). This method is only to be used in a room in which the temperature may be kept nearly constant. The directions for its use accompany the apparatus.

Other methods for the approximate estimation of proteid are the optical methods of CHRISTENSEN and MYGGE, of ROBERTS and STOLNIKOW as modified by BRANDBERG, with HELLER'S test, which has been simplified for practical purposes by MITTELBACH. The density methods of LANG, HUPPERT, and ZAHOR are also very good. In regard to these and other methods we refer to HUPPERT-NEUBAUER'S Harn-Analyse, 10. Aufl.

There is at present no trustworthy method for the quantitative estimation of proteoses and peptone in the urine.

Nucleoalbumin and Mucin. According to K. MÖRNER traces of urinary mucoids may pass into solution in the urine; otherwise normal urine contains no mucin. There is no doubt that there may be cases where true mucin appears in the urine; in most cases mucin has probably been mistaken for so-called nucleoalbumin. The occurrence, under some circumstances, of nucleoalbumin in the urine is not to be denied, as such substances occur in the renal and urinary passages; still in most cases this nucleoalbumin, as shown by K. MÖRNER [4] is of an entirely different kind.

Every urine, according to MÖRNER, contains a little proteid and in addition substances precipitating proteid. If the urine freed from salts by dialysis is shaken with chloroform after the addition of 1–2 p. m. acetic acid, a precipitate is obtained which acts like a nucleoalbumin. If the acid filtrate is treated with seralbumin, a new and similar precipitate is obtained due to the presence of a residue of the substance which precipitates proteids. The most important of these proteid-precipitating substances is chrondroitin-sulphuric acid and nucleic acid, although the latter appears to a much smaller extent. Taurocholic acid may in a few instances, especially in icteric urines, be precipitated. The substances isolated by different investigators from urine by the addition of acetic acid and called "dissolved mucin" or "nucleoalbumin" are considered by MÖRNER to be a combination of proteid chiefly with chrondroitin-sulphuric acid, and to a less extent with nucleic acid, and also perhaps with taurocholic acid.

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[1] Hammarster, Pflüger's Arch., 17; Hofmeister and Pohl, Arch. f. exp. Path. u. Pharm., 20.

[2] In regard to the literature on this meth. d and the numerous experiments to determine its value see Huppert-Neubauer, 10. Aufl. 853.

[3] Christensen, Virchow's Arch., 115.

[4] Skand. Arch. f. Physiol., 6.

As normal urine habitually contains an excess of substances capable of precipitating proteids, it is apparent that an increased elimination of so-called nucleoalbumin may be caused simply by an augmented excretion of proteid. This happens to a still greater extent in cases where the proteid as well as the proteid-precipitating substance is eliminated to an increased extent.

Detection of so-called Nucleoalbumins. When a urine becomes cloudy or precipitates on the addition of acetic acid, and when it gives a more typical reaction with HELLER's test after the dilution of the urine than before, one is justified in making tests for mucin and nucleoalbumin. As the salts of the urine interfere considerably with the precipitation of these substances by acetic acid, they must first be removed by dialysis. As large a quantity of urine as possible is dialyzed (with the addition of chloroform) until the salts are removed. Then acetic acid is added until it contains 2 p. m., and the mixture allowed to stand. The precipitate is dissolved in water by the aid of the smallest possible quantity of alkali and precipitated again. In testing for chrondroitin-sulphuric acid a part is warmed on the water-bath with about 5 per cent hydrochloric acid. If positive results are obtained on testing for sulphuric acid and a reducing substance, then chondroproteid was present. If a reducing substance can be detected but no sulphuric acid, then mucin is probably there. If it does not contain any sulphuric acid or reducing substance, a part of the precipitate is exposed to pepsin digestion and another part used for the determination of any organic phosphorus. If positive results are obtained from these tests, then nucleoalbumin and nucleoproteid must be differentiated by special tests for nuclein bases. No positive conclusion can be drawn except by using very large quantities of urine.

Nucleohistone. In a case of pseudoleucæmia A. JOLLES found a phosphorized protein substance which he considers as identical with nucleohistone. *Histone* is claimed to have been found in some cases by KREHL and MATTHES, and by KOLISCH and BURIAN.[1]

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[1] Jolles, Ber. d. deutsch. chem. Gesellsch., **30**; Krehl and Matthes, Deutsch. Arch. f. klin. Med., **54**; Kolisch and Burian, Zeitschr. f. klin. Med., **29**.

Blood and Blood-coloring Matters. The urine may contain blood from hemorrhage in the kidneys or other parts of the urinary passages (HÆMATURIA). In these cases, when the quantity of blood is not very small, the urine is more or less cloudy and colored reddish, yellowish-red, dirty red, brownish red, or dark brown. In recent hemorrhages, in which the blood has not decomposed, the color is nearer blood-red. Blood-corpuscles may be found in the sediment, sometimes also blood-casts and smaller or larger blood-clots.

In certain cases the urine contains no blood-corpuscles, but only dissolved blood-coloring matters, hæmoglobin, or, and indeed quite often, methæmoglobin (HÆMOGLOBINURIA). The blood-pigments appear in the urine under different conditions, as in dissolution of blood in poisoning with arseniuretted hydrogen, chlorates, etc., after serious burns, after transfusion of blood, and also in the periodic appearance of hæmoglobinuria with fever. In hæmoglobinuria the urine may also have an abundant grayish-brown sediment rich in proteid which contains the remains of the stromata of the red blood-corpuscles. In animals hæmoglobinuria may be produced by many causes which force free hæmoglobin into the plasma.

To detect blood in the urine, we make use of the microscope, the spectroscope, the guaiacum test, and HELLER'S or HELLER-TEICHMANN'S test.

Microscopic Investigation. The blood-corpuscles may remain undissolved for a long time in acid urine; in alkaline urine, on the contrary, they are easily changed and dissolved. They often appear entirely unchanged in the sediment; in some cases they are distended and in others unequally pointed or jagged like a thorn-apple. In hemorrhage of the kidneys a cylindrical clot is sometimes found in the sediment which is covered with numerous red blood-corpuscles, forming casts of the urinary passages. These formations are called *blood-casts.*

The *spectroscopic investigation* is naturally of very great value; and if it be necessary to determine not only the presence but also the kind of coloring-matter, this method is indispensable. In regard to the optical behavior of the various blood-pigments we must refer to Chapter VI.

Guaiacum Test. Mix in a test-tube equal volumes of tincture of guaiacum and old turpentine which has become strongly ozonized by the action of air under the influence of light. To this mixture, which must not have the slightest blue color, add the urine to be tested. In the presence of blood or blood-pigments, first a bluish-green and then a beautiful blue ring appears where the two liquids meet. On shaking the mixture it becomes more or less blue. Normal urine or one containing proteid does not give this reaction. According to LIEBERMANN[1] this reaction is brought about by the blood pigments acting as catalysators upon the organic peroxides existing in the turpentine, accelerating the decomposition of these and the active oxygen taken up by the guaiaconic acid which is oxidized to guaiac blue (guaiaconic acid ozonide). Urine containing pus, even when no blood is present, gives a blue color with these reagents; but in this case the tincture of guaiacum alone, without turpentine, is colored blue by the urine (VITALI[3]). This is at least true for a tincture that has been exposed for some time to the action of air and sunlight. The blue color produced by pus differs from that produced by blood-coloring matters by disappearing on heating the urine to boiling. A urine alkaline by decomposition must first be made faintly acid before performing the reaction. The turpentine should be kept exposed to sunlight, while the tincture of guaiacum must be kept in a dark glass bottle. These reagents to be of use must be controlled by a liquid containing blood. With positive results, however, this test is not absolutely decisive, because other bodies may give a similar reaction; but when properly performed it is so extremely delicate that when it gives negative results any other test for blood is superfluous.

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[1] Pflüger's Arch., **104**.

HELLER-TEICHMANN'S *Test.* If a neutral or faintly acid urine containing blood is heated to boiling, one always obtains a mottled precipitate consisting of proteid and hæmatin. If caustic soda is added to the boiling hot test, the liquid becomes clear and turns green when examined in thin layers (due to hæmatin alkali), and a red precipitate, appearing green by reflected light, re-forms, consisting of earthy phosphates and hæmatin. This reaction is called HELLER'S blood-test. If this precipitate is collected after a time on a small filter, it may be used for the hæmin test (see page 213). If the precipitate contains only a little blood-coloring matter with a larger quantity of earthy phosphates, then wash it with dilute acetic acid, which dissolves the earthy phosphates, and use the residue for the preparation of TEICHMANN'S hæmin crystals. If, on the contrary, the amount of phosphates is very small, then first add a little $CaCl_2$ solution to the urine, heat to boiling, and add simultaneously with the caustic potash some sodium-phosphate solution. In the presence of only very small quantities of blood, first make the urine very faintly alkaline with ammonia, add tannic acid, acidify with acetic acid, and use this precipitate in the preparation of the hæmin crystals (STRUVE[2]).

O. and R. ADLER[3] have recommended leucomalachite green or benzidine in the presence of peroxide and acetic acid as especially sensitive reagents for blood. We have no great experience thus far as to the mode of use of these reagents in urine investigations.

Hæmatoporphyrin. Since the occurrence of hæmatoporphyrin in the urine in various diseases has been made very probable by several investigators, such as NEUSSER, STOKVIS, MACMUNN, LE NOBEL, COPEMAN, and others,[4] SALKOWSKI has positively shown the presence of this pigment in the urine after sulphonal intoxication. It was first isolated in a pure

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[1] See Maly's Jahresber., 18.

[2] Zeitschr. f. anal. Chem., 11.

[3] Zeitschr. f. physiol. Chem., 41.

[4] A very complete index of the literature on hæmatoporphyrin in the urine may be found in R. Zoja, Su qualche pigmento di alcune urine, etc., in Arch. Ital. di. c in. Med., 1893.

crystalline state by Hammarsten[1] from the urine of insane women after sulphonal intoxication. According to Garrod and Saillet[2] traces of hæmatoporphyrin (Saillet's urospectrin) occur regularly in normal urines. It is also found in the urine during different diseases, although it occurs only in small quantities. It has been found in considerable quantities in the urine after the lengthy use of sulphonal.

Urine containing hæmatoporphyrin is sometimes only slightly colored, while in other cases, as for example, after the use of sulphonal, it is more or less deep red. In these last-mentioned cases the color depends, in greatest part, not upon the hæmatoporphyrin, but upon other red or reddish-brown pigments which have not been sufficiently studied.

In the detection of small quantities of hæmatoporphyrin proceed as suggested by Garrod. Precipitate the urine with a 10 per cent caustic-soda solution (20 c.c. for every 100 c.c. of urine). The phosphate precipitate containing the pigment is dissolved in alcohol-hydrochloric acid (15–20 c.c.) and the solution investigated by the spectroscope. In more exact investigations make the solution alkaline with ammonia, add enough acetic acid to dissolve the phosphate precipitate, shake with chloroform, which takes up the pigment, and test this solution with the spectroscope.

In the presence of larger quantities of hæmatoporphyrin the urine is first precipitated, according to Salkowski, with an alkaline barium-chloride solution (a mixture of equal volumes of barium-hydrate solution, saturated in the cold, and a 10 per cent barium-chloride solution), or, according to Hammarsten,[3] with a barium-acetate solution. The washed precipitate, which contains the hæmatoporphyrin, is allowed to stand some time at the temperature of the room with alcohol containing hydrochloric or sulphuric acid and then filtered. The filtrate shows the characteristic spectrum of hæmatoporphyrin in acid solution and gives the spectrum of alkaline hæmatoporphyrin after saturation with ammonia. If the alcoholic solution is mixed with chloroform and a large quantity of water added and carefully shaken, sometimes a lower layer of chloroform is obtained which contains very pure hæmatoporphyrin, while the upper layer of alcohol and water contains the other pigments besides some hæmatoporphyrin.

Other methods which have no advantage over this one of Garrod have been suggested by Riva and Zoja as well as Saillet.[4]

Baumstark[5] found in a case of leprosy two characteristic coloring-matters in the urine, "urorubrohæmatin" and "urofuscohæmatin," which, as their names indicate, seem to stand in close ralationship to the blood-coloring matters. *Urorubrohæmatin*, $C_{68}H_{94}N_8Fe_2O_{26}$, contains iron and shows in acid solution an absorption-band in front of *D* and a broader one back of *D*. In alkaline solution it shows four bands—behind *D*, at *E*, beyond *F*, and behind *G*. It is not soluble either in water, alcohol, ether, or chloroform. It gives a beautiful brownish-red non-dichroitic liquid with alkalies. *Urofuscohæmatin*, $C_{68}H_{106}N_8O_{26}$, which is free from iron, shows no characteristic spectrum; it dissolves in alkalies, producing a brown color. It remains to be proved whether these two pigments are related to (impure) hæmatoporphyrin.

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[1] Salkowski, Zeitschr. f. physiol. Chem., **15**; Hammarsten, Skand. Arch. f. Physiol., **3**.

[2] Garrod, Journ. of Physiol., **13** (contains review of literature) and **17**; Saillet, Revue de Médecine, **16**.

[3] Salkowski, l. c.; Hammarsten, l. c.

[4] Riva and Zoja, Maly's Jahresber., **24**; Saillet, l. c. See also Nebelthau, Zeitschr. f. physiol. Chem., **27**.

[5] Pflüger's Arch., **9**.

Melanin. In the presence of melanotic cancers dark pigments are sometimes eliminated with the urine. K. MÖRNER has isolated two pigments from such a urine, of which one was soluble in warm 50–75 per cent acetic acid, while the other, on the contrary, was insoluble. The one seemed to be *phymatorhusin* (see Chapter XVI). Usually the urine does not contain any melanin, but a chromogen of melanin, a *melanogen*. In such cases the urine gives EISLET'S reaction, becoming dark-colored with oxidizing agents, such as concentrated nitric acid, potassium bichromate, and sulphuric acid, as well as with free sulphuric acid. Urine containing melanin or melanogen is colored black by a ferric-chloride solution (v. JAKSCH [1]).

Urorosein, so named by NENCKI,[2] is a urinary coloring-matter occurring in various diseases, but which is not a constituent of normal urine. The pigment does not occur preformed in the urine, but first makes its appearance after the addition of mineral acids. It is readily soluble in water, dilute mineral acids, ethyl and amyl alcohol, and can be removed from the acid urine by shaking with the latter. It differs from indigo red in the following: Alkalies immediately decolorize a urorosein solution, but not an indigo-red solution. Urorosein is removed from its amyl-alcohol solution by shaking with dilute alkali, while indigo red is not. If the acid urine is shaken with chloroform, indigo red is taken up, but not urorosein. Urorosein is soon decomposed by light and shows a sharply defined absorption-band between *D* and *E*. The red pigment appearing in urines rich in skatol after the addition of hydrochloric acid differs from urorosein by being insoluble in water, but readily soluble in ether and chloroform. The statements in regard to the properties of skatol-red are somewhat divergent, and it is therefore difficult to state a positive difference between urorosein and skatol-red.

Pus occurs in the urine in various inflammatory affections, especially in catarrh of the badder and in inflammation of the pelvis of the kidneys or of the urethra.

Pus is best detected by means of the microscope. The pus-cells are rather easily destroyed in alkaline urines. In detecting pus we make use of DONNÉ'S pus test, which is performed in the following way: Pour off the urine from the sediment as carefully as possible, place a small piece of caustic alkali on the sediment, and stir. If the pus-cells have not been previously changed, the sediment is converted by this means into a slimy tough mass.

The pus-corpuscles swell up in alkaline urines, dissolve, or at least are so changed that they cannot be recognized under the microscope. The urine in these cases is more or less slimy or fibrous, and the proteid can be precipitated in large flakes by acetic acid, so that it might possibly be mistaken for mucin. The closer investigation of the precipitate produced by acetic acid, and especially the appearance or non-appearance of a reducing substance after boiling it with a mineral acid, demonstrates the nature of the precipitated substance. Urine containing pus always contains proteid.

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[1] K. Mörner, Zeitschr. f. physiol. Chem., **11**; v. Jaksch, *ibid.*, **13**.

[2] Nencki and Sieber, Journ. f. prakt. Chem. (N. F.), **26**.

Bile-acids. The reports in regard to the occurrence of bile-acids in the urine under physiological conditions do not agree. According to DRAGENDORFF and HÖNE traces of bile-acids occur in the urine; according to MACKAY and v. UDRÁNSZKY and K. MÖRNER[1] they do not. Pathologically they are present in the urine in hepatogenic icterus, although not invariably.

Detection of Bile-acids in the Urine. PETTENKOFER'S test gives the most decisive reaction; but as it gives similar color reactions with other bodies, it must be supplemented by the spectroscopic investigation. The direct test for bile-acids is easily performed after the addition of traces of bile to a normal urine. But the direct detection in a colored icteric urine is more difficult and gives very misleading results; the bile-acid must therefore always be isolated from the urine. This may be done by the following method of HOPPE-SEYLER, which is slightly modified in non-essential points.

HOPPE-SEYLER'S METHOD. Concentrate the urine and extract the residue with strong alcohol. The filtrate is freed from alcohol by evaporation and then precipitated by basic lead acetate and ammonia. The washed precipitate is treated with boiling alcohol, filtered hot, the filtrate treated with a few drops of soda solution, and evaporated to dryness. The dry residue is extracted with absolute alcohol, filtered, and an excess of ether added. The amorphous or, after a longer time, crystalline precipitate consisting of the alkali salts of the biliary acids is used in performing PETTENKOFER'S test.

HAYCRAFT has suggested a reaction for clinical purposes which consists in sprinkling flowers of sulphur upon the urine. In icteric urine the powder quickly sinks to the bottom, while in normal urine it remains on the surface. The value of this test is still questioned.

Bile-pigments occur in the urine in different forms of icterus. A urine containing bile-pigments is always abnormally colored—yellow, yellowish brown, deep brown, greenish yellow, greenish brown, or nearly pure green. On shaking it froths and the bubbles are yellow or yellowish green in color. As a rule icteric urine is somewhat cloudy, and the sediment is frequently, especially when it contains epithelium-cells, rather strongly colored by the bile-pigments. In regard to the occurrence of urobilin in icteric urine see p. 604.

Detection of Bile-coloring Matters in Urine. Many tests have been proposed for the detection of these substances. Ordinarily we obtain the best results either with GMELIN'S or with HUPPERT'S test.

GMELIN'S *test* may be applied directly to the urine; but it is better to use ROSENBACH'S modification. Filter the urine through a very small filter, which becomes deeply colored from the retained epithelium-cells and bodies of that nature. After the liquid has entirely passed through apply to the inside of the filter a drop of nitric acid which contains only very little nitrous acid. A pale-yellow spot will be formed which is surrounded by colored rings which appear yellowish red, violet, blue, and green from within outward. This modification is very delicate, and it is hardly possible to mistake indican and other coloring-matters for the bile-pigments. Several other modifications of GMELIN's direct test, e. g., with concentrated sulphuric acid and nitrate, etc., have been proposed, but they are neither simpler nor more delicate than ROSENBACH's modification.

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[1] Cited from Huppert-Neubauer, Harn-Analyse, 10. Aufl. 229.

HUPPERT's *Reaction*. In a dark-colored urine or one rich in indican good results are not always obtained with GMELIN's test. In such cases, as also in urines containing blood-coloring matters at the same time, the urine is treated with lime-water, or first with some $CaCl_2$ solution, and then with a solution of soda or ammonium carbonate. The precipitate which contains the bile-coloring matters is filtered, washed, dissolved in alcohol which contains 5 c.c. of concentrated hydrochloric acid in 100 c.c. (I. MUNK), and heated to boiling when the solution becomes green or bluish green. According to NAKAYAMA [1] this reaction is more delicate on using a mixture of ferric chloride, acid, and alcohol.

HAMMARSTEN's *Reaction*. For ordinary cases it is sufficient to add a few drops of urine to about 2–3 c.c. of the reagent (see page 322), when the mixture immediately after shaking turns a beautiful green or bluish green, which color remains for several days. In the presence of only very small quantities of bile-pigments, especially when blood or other pigments are simultaneously present, pour about 10 c.c. of the acid or nearly neutral (not alkaline) urine into the tube of a small centrifugal machine and add $BaCl_2$ solution and centrifuge for about one minute. The liquid is decanted off and the sediment stirred with about 1 c.c. of the reagent and centrifuged again. A beautiful green solution is obtained, which may be changed by the addition of increased quantities of the acid mixture to blue, violet, red, and reddish yellow. The green color may be obtained in the presence of 1 part bile-pigment in 500,000–1,000,000 parts urine. In the presence of large amounts of other pigments calcium chloride is better suited than barium chloride.

BOUMA [2] has suggested the use of alcohol containing ferric chloride and hydrochloric acid instead of the above-mentioned acid mixture. He has also worked out a colorimetric method of quantitative estimation of bilirubin in urine by means of this reagent.

The very delicate reaction suggested by JOLLES is unfortunately not serviceable on account of the formation of froth, especially in the presence of proteid and blood-pigments; but he has changed it by centrifuging the urine with chloroform and barium chloride and suspending the chloroform-barium residue in alcohol; after which he treats it with a solution of iodine and mercuric chloride in alcohol containing hydrochloric acid.[3] The color becomes green or bluish green. This test seems to be good.

STOKVIS's *reaction* is especially valuable as a control test in those cases in which the urine contains only very little bile-coloring matter together with larger quantities of other coloring-matters. The test is performed as follows: 20–30 c.c. of urine is treated with 5–10 c.c. of a solution of zinc acetate (1:5). The precipitate is washed on a small filter with water and then dissolved in a little ammonia. The new filtrate gives, either directly or after it has stood a short time in the air until it has a peculiar brownish-green color, the absorption-bands of bilicyanin (see page 322). This reaction is unfortunately not sufficiently delicate.

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[1] Munk, Arch. f. (Anat. u.) Physiol., 1898; Nakayama, Zeitschr. f. physiol. Chem., 36

[2] Deutsch. med. Wochenschr., 1902 and 1904.

[3] Deutsch. Arch. f. klin. Med., 78.

Many other reactions for bile-coloring matters in the urine have been proposed; but as those above mentioned are sufficient, it is perhaps only necessary to give here a few of the other reactions without entering into details.

SMITH'S *Reaction.* Pour carefully over the urine some tincture of iodine, whereby a green ring appears between the two liquids. The urine may also be shaken with the tincture of iodine until it has a green color.

EHRLICH'S *Test.* First mix the urine with an equal volume of dilute acetic acid and then add drop by drop a solution of sulphodiazobenzene. The acid mixture becomes dark red in the presence of bilirubin, and this color becomes bluish violet on the addition of glacial acetic acid. The sulphodiazobenzene is prepared by mixing 1 gram of sulphanilic acid, 15 c.c. of hydrochloric acid, and 0.1 gram of sodium nitrite; this solution is diluted to 1 liter with water. This test is not successful and positive when directly applied, if the urine is rich in other pigments.

MEDICINAL COLORING-MATTERS produced from santonin, rhubarb, senna, etc., may give an abnormal color to the urine and may be mistaken for bile-pigments, or, in alkaline urines, perhaps for blood-coloring matters. If hydrochloric acid is added to such a urine, it becomes yellow or pale yellow, while on the addition of an excess of alkali it takes on a more or less beautiful red color.

Sugar in Urine.

The occurrence of traces of dextrose in the urine of perfectly healthy persons has been, as above stated (page 608), quite positively proved. If sugar appears in the urine in constant and especially in large quantities, it must be considered as an abnormal constituent. In a previous chapter several of the principal causes of glycosuria in man and animals were mentioned, and the reader is referred to Chapters VIII and IX for the essential facts in regard to the appearance of sugar in the urine.

In man the appearance of dextrose in the urine has been observed under various pathological conditions, such as lesions of the brain and especially of the medulla oblongata. abnormal circulation in the abdomen, diseases of the heart, lungs and liver, cholera, and many other diseases. The continued presence of sugar in human urine. sometimes in very considerable quantities, occurs in DIABETES MELLITUS. In this disease there may be an elimination of 1 kilogram or even more of dextrose per day. In the beginning of the disease. when the quantity of sugar is still very small, the urine often does not appear abnormal. In the more developed, typical cases the quantity of urine voided increases considerably, to 3–6–10 liters per day. The percentage of the physiological constituents is as a rule very low, while their absolute daily quantity is increased. The urine is pale, but of a high specific gravity, 1.030–1.040 or even higher. The high specific gravity depends upon the quantity of sugar present, which varies in different cases, but may reach 10 per cent. The urine is therefore characterized in typical cases of diabetes by the very large quantity voided, by the pale color and high specific gravity, and by its containing sugar.

That the urine after the introduction into the system of certain medicinal agents or poisonous bodies contains reducing substances, conjugated glucuronic acids, which may be mistaken for sugar, has already been mentioned.

The properties and reactions of dextrose have been considered in a previous chapter, and it remains but to mention the methods for the detection and quantitative determination of dextrose in the urine.

The *detection of sugar* in the urine is ordinarily, in the presence of not too small quantities, a very simple task. The presence of only very small quantities may make its detection sometimes very difficult and laborious. A urine containing proteid must first have the proteid removed by coagulation with acetic acid and heat before it can be tested for sugar.

The tests which are most frequently employed and are especially recommended are as follows:

TROMMER'S *Test.* In a typical diabetic urine or one rich in sugar this test succeeds well, and it may be performed in the manner suggested on page 116. This test may lead to very great mistakes in urines poor in sugar, especially when they have at the same time normal or increased amounts of physiological constituents, and therefore it cannot be recommended to physicians or to persons inexperienced in such work. Normal urine contains reducing substances, such as uric acid, creatinine, and others, and therefore a reduction takes place in all urines on using this test. A separation of copper suboxide does not generally occur, but still if one varies the proportion of the alkali to the copper sulphate and boils, there takes place an actual separation of suboxide in normal urines, or a peculiar yellowish red liquid due to finely divided cuprous hydrate. This occurs especially on the addition of much alkali or too much copper sulphate, and by careless manipulation the inexperienced worker may therefore sometimes obtain apparently positive results in a normal urine. On the other hand, as the urine contains substances, such as creatinine and ammonia (from the urea), which in the presence of only a little sugar may keep the copper suboxide in solution, the investigator may easily overlook small quantities of sugar that may be present.

TROMMER'S test may of course be made positive and useful, even in the presence of very small amounts of sugar, by using the modification suggested by WORM MÜLLER. As this modification is rather complicated and requires much practice and exactness, it is probably rarely employed by the busy physician. The following test is to be preferred.

ALMÉN'S *bismuth test*, which recently has been incorrectly called NYLANDER'S test, is performed with the alkaline-bismuth solution prepared as above described (page 116), For each test 10 c.c. of urine is taken and treated with 1 c.c. of the bismuth solution and boiled for a few minutes. In the presence of sugar the urine becomes dark yellow or yellowish brown. Then it grows darker, cloudy, dark brown, or nearly black, and non-transparent. After a longer or shorter time a black deposit appears, the supernatant liquid gradually clears, but still remains colored. In the presence of only very little sugar the test does not become black or dark brown, but simply deeper-colored, and not until after some time is there seen on the upper layer of the phosphate precipitate a dark or black layer (of bismuth?). In the presence of much sugar a larger amount of the reagent may be used without disadvantage. In a urine poor in sugar only 1 c.c. of the reagent for every 10 c.c. of the urine must be employed.

Small amounts of proteid may retard this reaction and reduce the delicacy of the test. Large quantities of proteid may, however, give rise to an error by forming bismuth sulphide, and therefore it must always be first removed. The statement of BECHHOLD that mercury compounds in the urine disturb the test has not been substantiated by ZEIDLITZ[1] on properly performing the test. Those sources of error which in TROMMER'S test are caused by the presence of uric acid and creatinine are removed by using this test. The bismuth test is, moreover, readily performed, and on this account is to be recommended to the physician.

The bumping and ejection of the fluid can be readily prevented by heating over a very small flame after the test has been brought to a boil and by gently shaking the contents of the not too narrow test-tube. The recommendation of heating for a longer time in the water-bath, fifteen minutes or more, is to be discarded, as the delicacy of the test is thereby so much increased that it gives a reaction with a physiological sugar content of 0.02 per cent.

When the amount of sugar in the urine is not less than 0.1 per cent a positive reaction is obtained if the test is boiled for 2–3 minutes and then allowed to stand quietly for 5 minutes. The phosphate precipitate is then black or nearly black. In detecting smaller quantities of sugar—0.05 per cent, the test as a rule must be boiled longer—about 5 minutes.

The value of this test lies in the fact that it positively detects small quantities of sugar—0.1 per cent or somewhat less. Equally with TROMMER'S test it is a reduction test; and shows also certain other reducing bodies besides the sugar. These bodies are certain conjugated glucuronic acids which may appear in the urine. After the use of certain therapeutic agents, such as rhubarb, senna, antipyrine, salol, turpentine and others, the bismuth test gives positive results. From this it follows that we should never be satisfied with this test alone, especially when the reduction is not very great. When this test gives negative results the urine can be considered from a clinical standpoint as free from sugar, and when it gives positive results other tests must be applied. Among these the fermentation test and the polarization test are of special value.

The question in what degree the use of therapeutic agents affects the WORM-MÜLLER test has been only slightly investigated. That normal urines sometimes give the bismuth test is a common experience, and these urines according to the experience of HAMMARSTEN and certain other observers, as a rule also give the WORM-MÜLLER test when properly performed. There does not seem to be any doubt that in many of these cases it is due to an increased quantity of physiological sugar in the urine. Further investigations on this point are very desirable.

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[1] Bechhold, Zeitschr. f. physiol. Chem., **46**; Zeidlitz, unpublished investigations.

Fermentation Test. On using this test the process must vary according as the bismuth test shows small or large quantities of sugar. If a rather strong reduction is obtained, the urine may be treated with yeast and the presence of sugar determined by the generation of carbon dioxide. In this case the acid urine, or that faintly acidified with a little sulphuric or hydrochloric acid, is treated with compressed yeast or yeast which has previously been washed by decantation with water. Pour this urine to which the yeast has been added into a SCHRÖTTER's gas-burette or a LOHNSTEIN's saccharimeter (see below). As the fermentation proceeds, the carbon dioxide collects in the upper part of the tube, while a corresponding quantity of liquid is expelled below. As a control in this case two similar tests must be made, one with normal urine and yeast to learn the quantity of gas usually developed, and the other with a sugar solution and yeast to determine the activity of the yeast.

If, on the contrary, only a faint reduction with the bismuth test is found, no positive conclusion can be drawn from the absence of any carbon dioxide or the appearance of a very insignificant quantity. The urine absorbs considerable amounts of carbon dioxide, and in the presence of only small amounts of sugar the fermentation test as above performed may lead to negative or inaccurate results. In this case proceed in the following way: Treat the acid urine, or urine which has been faintly acidified with a little sulphuric acid, with yeast whose activity has been tested by a special test on a sugar solution, and allow it to stand 24–30 hours at about 30°. Then test again with the bismuth test, and if the reaction now gives negative results, then sugar was previously present. But if the reaction continues to give positive results, then it shows, if the yeast is active, the presence of other reducing, unfermentable substances.

In performing the fermentation test care should be taken that the urine be acid before as well as after fermentation. If the reaction becomes alkaline during fermentation (alkaline fermentation), then the test must be discarded. The vessel must be perfectly clean and strongly heated before use. To make sure the urine may be boiled before fermentation.[1]

If a good polariscope is at hand it must not be forgotten to control the results of the fermentation by determining the rotation before and after fermentation. The phenylhydrazine test also, in many otherwise doubtful cases, gives good service in testing urines for sugar.

Phenylhydrazine Test. According to v. JAKSCH this test is performed in the following way: Add in a test-tube containing 6–8 c.c. of the urine two knife-points of phenylhydrazine hydrochloride and three knife-points of sodium acetate, and when the salts do not dissolve on warming add more water. The test-tube is placed in boiling water and warmed on the water-bath. It is then placed in a beaker of cold water. If the quantity of sugar present is not too small, a yellow crystalline precipitate is now obtained. If the precipitate appears amorphous, there are found, on looking at it under the microscope, yellow needles singly and in groups. If very little sugar is present, pour the test into a conical glass and examine the sediment. In this case at least a few phenylglucosazone crystals are found, while the occurrence of larger and smaller yellow plates or highly refractive brown globules does not show the presence of sugar. This reaction is very reliable, and by it the presence of 0.03 per cent sugar can be detected (ROSENFELD, GEYER[1]). In doubtful cases where certainty is desired, prepare the crystals from a large quantity of urine, dissolve them on the filter by pouring over them hot alcohol, treat the filtrate with water, and boil off the alcohol. Still better, the precipitate is dissolved, according to NEUBERG, in some pyridine, and again precipitated as crystals by the addition of benzene, ligroin, or ether. If the characteristic yellow crystalline needles, whose melting-point (204–205° C.) may also be determined, are now obtained, then this test is decisive for the presence of sugar. It must not be forgotten that levulose gives the same osazone as dextrose, and that a further investigation is necessary in certain cases.

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[1] On the performance of the fermentation test and certain sources of error, see Salkowski, Berlin. klin. Wochenschr., 1905 (Ewald-Festnummer), and Pflüger, Pflüger's Arch., **105**.

The following modification by A. NEUMANN[2] is simple, practical, and at the same time sufficiently delicate. 5 c.c. of the urine is treated with 2 c.c. of acetic acid (30 per cent) saturated with sodium acetate, 2 drops of pure phenylhydrazine added, and the mixture boiled in a test-tube until it measures 3 c.c. After quickly cooling warm again and then allow it to cool slowly. After 5–10 minutes beautifully formed crystals are obtained even in the presence of only 0.02 per cent sugar. According to the experience of HAMMARSTEN this modification, even in the presence of 0.1 per cent sugar in concentrated urines, does not always give a positive reaction.

The value of the phenylhydrazine test has been considerably debated, and the objection has been made that glucuronic acids also give a similar precipitate. A confounding with glucuronic acid is, according to HIRSCHL, not to be apprehended when the test is not heated in the water-bath for too short a time (one hour). KISTERMANN found this precaution insufficient, and ROOS states that the phenylhydrazine test always gives a positive result with human urine, which coincides with E. HOLMGREN'S[3] and HAMMARSTEN'S experience. This test only shows a non-physiological quantity of sugar when a rather abundant crystallization is obtained from a small quantity of urine (about 5 c.c.). Too great a delicacy of test is not to be recommended.

RUBNER'S *test* is performed as follows: The urine is precipitated by an excess of a concentrated lead-acetate solution and the filtrate carefully treated with enough ammonia to produce a flocculent precipitate. It is then heated to boiling, when the precipitate becomes flesh-colored or pink in the presence of sugar.

Polarization. This test is of great value, especially as in many cases it quickly differentiates between dextrose and other reducing, sometimes levogyrate, substances, such as the conjugated glucuronic acids. In the presence of only very little sugar the value of this test depends on the delicacy of the instrument and the dexterity of the observer. As a urine which shows no rotation or is actually faintly levorotatory, may contain 0.2 per cent sugar or perhaps even more, this test must be combined with the fermentation test if we are seeking very small amounts of sugar. The sugar in these cases can be detected only by the use of a very accurate and delicate instrment. This method is in many cases not serviceable for the physician.

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[1] Rosenfeld, Deutsch. med. Wochenschr., 1888; Geyer, cited from Roos, Zeitschr. f. physiol. Chem., **15**.

[2] Arch. f. (Anat. u.) Physiol., 1899, Suppl. See also Margulies, Berlin. klin. Wochenschr., 1900.

[3] Hirschl, Zeitschr. f. physiol. Chem., **14**; Kistermann, Deutsch. Arch. f. klin. Med., **50**; Roos, l. c.; Holmgren, Maly's Jahresber., **27**.

If small quantities of sugar are to be isolated from the urine, precipitate the urine first with sugar of lead, filter, precipitate the filtrate with ammoniacal basic lead acetate, wash this precipitate with water, decompose it with H_2S when suspended in water, concentrate the filtrate, treat it with strong alcohol until it is 80 vol. per cent, filter when necessary, and add an alcoholic caustic-alkali solution. Dissolve the precipitate consisting of saccharates in a little water, precipitate the potash by an excess of tartaric acid, neutralize the filtrate with calcium carbonate in the cold, and filter. The filtrate may be used for testing with the polariscope as well as for the fermentation, bismuth, and phenylhydrazine tests. The presence of dextrose may be detected by this same process in animal fluids or tissues from which the proteids have been removed by coagulation or by the addition of alcohol.

In the isolation of sugar and carbohydrates from the urine the benzoic-acid esters of the same may be prepared according to BAUMANN's method. The urine is made alkaline with caustic soda to precipitate the earthy phosphates, the filtrate treated with 10 c.c. of benzoyl chloride and 120 c.c. of 10 per cent caustic-soda solution for every 100 c.c. of the filtrate (REINBOLD[1]), and shaken until the odor of benzoyl chloride has disappeared. After standing sufficiently long the ester is collected, finely divided, and saponified with an alcoholic solution of sodium ethylate in the cold according to BAISCH's method,[2] and the various carbohydrates separated according to his suggestion.

To the physician, who naturally wants simple and quick methods, the bismuth test is especially to be recommended. If this test gives negative results, the urine is to be considered as free from sugar in a clinical sense. If it gives positive results, the presence of sugar must be controlled by other tests, especially by the fermentation test.

Other tests for sugar, as, for example, the reaction with orthonitrophenylpropiolic acid, picric acid, diazobenzene-sulphonic acid, are superfluous. The reaction with α-naphthol, which is a reaction for carbohydrates in general, for glucuronic acid and mucin, may, because of its extreme delicacy, give rise to mistakes, and is therefore not to be recommended to physicians. Normal urines give this test, and if the strongly diluted urine gives the reaction the presence may be suspected of great quantities of carbohydrates. In these cases more positive results are obtained by using other tests. This test requires great cleanliness, and it has the inconvenience that sufficiently pure sulphuric acid is not always readily procurable. Several investigators, such as v. UDRÁNSKY, LUTHER, ROOS and TREUPEL,[3] have investigated this test in regard to its applicability as an approximate test for carbohydrates in the urine.

Quantitative Determination of Sugar in the Urine. The urine for such an estimation must fisrt be tested for proteid, and if any be present it must be removed by coagulation and the addition of acetic acid, care being taken not to increase or diminish the original volume of urine. The quantity of sugar may be determined by TITRATION with FEHLING'S or KNAPP'S solutoin, by FERMENTATION, by POLARIZATION, and in other ways.

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[1] Pflüger's Arch., **91**.

[2] Zeitschr. f. physiol. Chem., **19**.

[3] See Roos and Treupel, Zeitschr. f. physiol. Chem., **15** and **16**.

The titration liquids not only react with sugar, but also with certain other reducing substances, and on this account the titration methods give rather high results. When large quantities of sugar are present, as in typical diabetic urine, which generally contains a lower percentage of normal reducing constituents, this is indeed of little account; but when small quantities of sugar are present in an otherwise normal urine, the mistake may, on the contrary, be important, as the reducing power of normal urine may correspond to 5 p. m. dextrose (see page 609). In such cases the titration procedure must be employed in connection with the fermentation method, which will be described later. It is to be remarked that in typical diabetic urines with considerable quantities of sugar the titration with FEHLING'S solution is just as reliable as with KNAPP'S solution. When the urine on the other hand, contains only little sugar with normal amounts of physiological constituents, then the titration with FEHLING'S solution is more difficult, in certain cases indeed almost impossible, and the results become very uncertain. In such cases KNAPP'S method gives good results, according to WORM MÜLLER and his pupils.[1]

The TITRATION with FEHLING'S SOLUTION depends on the power of sugar to reduce copper oxide in alkaline solutions. For this there was formerly employed a solution which contained a mixture of copper sulphate, Rochelle salt, and sodium or potassium hydrate (FEHLING'S solution); but as such a solution readily changes, use is made of a copper-sulphate solution and an alkaline Rochelle-salt solution prepared separately, and the two solutions mixed in equal volumes before using.

The concentration of the copper-sulphate solution is such that 10 c.c. of this solution is reduced by 0.05 gram of dextrose. The copper-sulphate solution contains 34.65 grams of pure, crystallized, non-efflorescent copper sulphate in 1 liter. The sulphate is crystallized from a hot saturated solution by cooling and stirring, and the crystals are separated from the mother-liquor and pressed between blotting-paper until dry. The Rochelle-salt solution is prepared by dissolving 173 grams of the salt in 350 c.c. of water, adding 600 c.c. of a caustic-soda solution of a specific gravity of 1.12, and diluting with water to 1 liter. According to WORM MÜLLER, these three liquids—Rochelle-salt solution, caustic soda, and water—should be separately boiled before mixing together. For each titration mix in a small flask or porcelain dish exactly 10 c.c. of the copper-sulphate solution and 10 c.c. of the alkaline Rochelle-salt solution and add 30 c.c. of water.

The urine, freed from proteid, is diluted with water before the titration, so that 10 c.c. of the copper solution requires between 5 and 10 c.c. of the diluted urine, which corresponds to between 1 per cent and ½ per cent of sugar. A urine of a specific gravity of 1.030 may be diluted five times; one more concentrated, ten times. The urine so diluted is poured into a burette and allowed to flow into the boiling copper-sulphate and Rochelle-salt solution until the copper oxide is completely reduced. This has taken place when, immediately after boiling, the blue color of the solution disappears. It is very difficult and requires some practice to exactly determine this point, especially when the copper suboxide settles with difficulty. To determine whether the color has disappeared, allow the copper suboxide to settle a little below the meniscus formed by the surface of the liquid. If this layer is not blue, the operation is repeated, adding 0.1 c.c. less of urine; and if, after the copper suboxide has settled, the liquid has a blue color, the titration may be considered as completed. Because of the difficulty in obtaining this point exactly another end-reaction has been suggested. This consists in filtering immediately after boiling a small portion of the titrated mixture through a small filter into a test-tube which contains a little acetic acid and a few drops of potassium-ferrocyanide solution and water. The smallest quantity of copper is shown by a red coloration. If the operation is quickly conducted so that no oxidation of the suboxide into oxide takes place, this end-reaction is of value for urines which are rich in sugar and poor in urea and which have been strongly diluted with water. In urines poor in sugar which contain the normal amount of urea and which have not been considerably diluted, a considerable quantity of ammonia may be formed from the urea on boiling the alkaline liquid. This ammonia dissolves the suboxide in part, which then easily passes into oxide; besides the dissolved suboxide gives a red color with potassium ferrocyanide. In just those cases in which the titration is most difficult this end-reaction is the least reliable. Practice also renders it unnecessary, and it is therefore best to depend simply upon the appearance of the liquid.

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[1] Pflüger's Arch., 16 and 23; Otto, Journal f. prakt. Chem. (N. F.), 26.

To facilitate the settling of the copper suboxide and thereby clearing the liquid, MUNK[1] has suggested the addition of a little calcium-chloride solution and boiling again. A precipitate of calcium tartrate is produced which carries down the suspended copper suboxide with it, and the color of the liquid can then be seen more readily. This artifice succeeds in many cases, but unfortunately there are urines in which the titration wtih FEHLING's solution in no way gives exact results. In those cases in which only small quantities of sugar exist in a urine rich in physiological constituents it is best to dissolve a very exactly weighed quantity of pure dextrose or dextrose-sodium chloride in the urine. The urine can now be strongly diluted with water and the titration becomes successful. The difference between the sugar added and that found by titration gives the reducing power of the original urine calculated as dextrose.

The necessary conditions for the success of the titration under all circumstances are, according to SOXHLET,[2] the following: The copper-sulphate and Rochelle-salt solution must, as above, be diluted to 50 c.c. with water; the urine should contain only between 0.5 and 1 per cent of sugar, and the total quantity of urine required for the reduction must be added to the titration liquid at once and boiled with it. From this last condition it follows that the titration is dependent upon minute details, and several titrations are required for each determination.

It is best to give here an example of the titration. The proper amount of copper-sulphate and Rochelle-salt solution and water (total volume = 50 c.c.) is heated to boiling in a flask; the color must remain blue. The urine diluted five times is now added to the boiling-hot liquid, 1 c.c. at a time; after each addition of urine boil for a few seconds and look for the appearance of the end-reaction. If one finds, for example, that 3 c.c. is too little, but that 4 c.c. is too much (the liquid becoming yellowish), then the urine has not been sufficiently diluted, for it should require between 5 and 10 c.c. of the urine to produce the complete reduction. The urine is now diluted ten times, and it should require between 6 and 8 c.c. for a total reduction. Now prepare four new tests, which are boiled simultaneously to save time, and add at one time respectively 6, 6½, 7, and 7½ c.c. of urine. If it is found that between 6½ and 7 c.c. are necessary to produce the end-reaction, then make four other tests, to which add respectively 6.6, 6.7, 6.8, and 6.9 c.c. of urine. If in this case the liquid is still somewhat bluish with 6.7 c.c. and completely decolorized with 6.8 c.c., the average figure 6.75 c.c. is considered as correct.

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[1] Virchow's Arch., **105**.

[2] Journ. f. prakt. Chem. (N. F.), **21**.

The calculation is simple. The 6.75 c.c. used contains 0.05 gram of sugar, and the percentage of sugar in the dilute urine is therefore $(6.75:0.05=100:x)=\frac{5}{6.75}=0.74$. But as the urine was diluted with ten times its volume of water, the undiluted urine contained $\frac{5\times 10}{6.75}=7.4$ per cent. The general formula on using 10 c.c. of copper-sulphate solution is therefore $\frac{5\times n}{k}$, in which n represents the number of times the urine has been diluted, and k the number of cubic centimeters of the diluted urine employed for the titration.

The **titration according to Knapp** depends on the fact that mercuric cyanide in alkaline solution is reduced to metallic mercury by dextrose. The titration liquid should contain 10 grams of chemically pure dry mercuric cyanide and 100 c.c. of caustic-soda solution of a specific gravity of 1.145 per liter. When the titration is performed as described below (according to Worm-Müller and Otto), 20 c.c. of this solution should correspond to exactly 0.05 gram of dextrose. If the process is carried out in other ways, the value of the solution is different.

In this titration, also, the quantity of sugar in the urine should be between ½ and 1 per cent and the extent of dilution necessary be determined by a preliminary test. To determine the end-reaction as described below, the test for the excess of mercury is made with sulphuretted hydrogen.

In performing the titration allow 20 c.c. of Knapp's solution to flow into a flask and dilute with 80 c.c. of water, or when the urine contains less than 0.5 per cent of sugar use only 40–60 c.c. After this heat to boiling and allow the dilute urine to flow gradually into the hot solution, at first 2 c.c., then 1 c.c., then 0.5 c.c., then 0.2 c.c., and lastly 0.1 c.c. After each addition let it boil ½ minute. When the end-reaction is approaching, the liquid begins to clarify and the mercury separates with the phosphates. The end-reaction is determined by taking a drop of the upper layer of the liquid into a capillary tube and then blowing it out on pure white filter-paper. The moist spot is first held over a bottle containing fuming hydrochloric acid and then over strong sulphuretted hydrogen. The presence of a minimum quantity of mercury salt in the liquid is shown by the spot becoming yellowish, which is best seen when it is compared with a second spot that has not been exposed to the gas. The end-reaction is still clearer when a small part of the liquid is filtered, acidified with acetic acid, and tested with sulphuretted hydrogen (**Otto**[1]). The calculations are just as simple as for the previous method.

This titration, unlike the previous one, may be performed equally well by daylight and by artificial light. **Knapp's** method has the following advantages over **Fehling's** method: It is applicable even when the quantity of sugar in the urine is very small and that of the other urinary constituents is normal. It is more easily performed, and the titration liquids may be kept without decomposing for a long time (**Worm Müller** and his pupils[2]). The views of the various investigators on the value of this titration method are nevertheless somewhat contradictory.

The titration according to **Pavy** consists in adding a boiling ammoniacal solution of copper sulphate to the urine until it is decolorized, when the suboxide formed is dissolved by the ammonia into a colorless solution. The admission of air must be completely excluded. In regard to the performance of this highly recommended method we must refer to the works of **Pavy**, **Kumagawa** and **Suto**, and **Sahli**.[3]

Besides the above-described methods there are various others. **K. B. Lehmann** uses an excess of copper salt and retitrates with potassium iodide and hyposulphite. The sugar can also be determined according to **Allihn**, and especially according to **Pflüger's** modification of this method.[4]

Estimation of the Quantity of Sugar by Fermentation. This may be done in various ways; the simplest method, and one at the same time sufficiently exact for ordinary cases, is that of **Roberts**. This consists in determining the specific gravity of the urine before and after fermentation. In the fermentation of sugar, carbon dioxide and alcohol are formed as chief products and the specific gravity is lowered, partly on account of the disappearance of the sugar and partly on account of the production of alcohol. **Roberts** found that a decrease of 0.001 in the specific gravity corresponded to 0.23 per cent sugar, and this has been substantiated since by several other investigators (**Worm Müller** and others). If the urine, for example, has a specific gravity of 1.030 before fermentation and 1.008 after, then the quantity of sugar contained therein was $22 \times 0.23 = 5.06$ per cent.

In performing this test the specific gravity must be taken at the same temperature before and after the fermentation. The urine must be faintly acid, and when necessary it should be acidified with a little hydrochloric acid or sulphuric acid. The activity of the yeast must, when necessary, be controlled by a special test. Place 200 c.c. of the urine in a 400 c.c. flask, add a piece of compressed yeast the size of a pea, and subdivide the yeast through the liquid by shaking; close the flask with a stopper provided with a finely-drawn-out glass tube, and allow the test to stand at the temperature of the room or, still better, at 30–35° C. After 24 hours the fermentation is ordinarily ended, but this must be verified by the bismuth test. After complete fermentation filter through a dry filter, bring the filtrate to the proper temperature, and determine the specific gravity.

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[1] Journal f. prakt. Chem., **26**.

[2] Pflüger's Arch., **16** and **23**.

[3] Pavy, The Physiology of the Carbohydrates, London, 1894; Kumagawa and Suto, Salkowski's Festschrift, 1904; Sahli, Deutsch. med. Wochenschr., 1905. In regard to other methods, see Huppert-Neubauer, Analyse des Harnes.

[4] Lehmann, Arch. f. Hygiene, **30**; Pflüger, Pflüger's Arch., **66**.

If the specific gravity be determined with a good pyknometer supplied with a thermometer and an expansion-tube, this method, when the quantity of sugar is not less than 4–5 p. m., gives, according to WORM MÜLLER, very exact results, but this has been disputed by BUDDE.[1] For the physician the method in this form is not quite serviceable. Even when the specific gravity is determined by a delicate urinometer which can give the density to the fourth decimal, quite exact results are not obtained, because of the ordinary errors of the method (BUDDE); but the errors are usually smaller than those which occur in titrations made by unskilled hands.

When the quantity of sugar is less than 5 p. m. these methods cannot be used. Such small amounts cannot, as already mentioned, be determined by titration directly, because the reducing power of normal urine corresponds to 4–5 p. m. of sugar. In such cases, according to WORM MÜLLER, it is better first to determine the reduction power of the urine by titration with KNAPP's solution, then ferment the urine with the addition of yeast and titrate again with KNAPP's solution. The difference found between the two titrations calculated as sugar gives the true quantity of the latter.

The determination of the sugar by fermentation can be so performed that the loss in weight due to the CO_2 can be estimated or the volume of the gas measured. For this last purpose LOHNSTEIN[2] has constructed a special fermentation saccharometer, of which his "precision saccharometer" is to be recommended. Based upon LOHNSTEIN's instrument, WAGNER[3] has constructed a ' fermentation saccharo-manometer," which has certain advantages over LOHNSTEIN's apparatus.

ESTIMATION OF SUGAR BY POLARIZATION. In this method the urine must be clear, not too deeply colored, and, above all, must not contain any other optically active substances besides dextrose. The urine may contain several levorotatory substances such as proteids, β-oxybutyric acid, conjugated glucuronic acids, the so-called LEO's sugar, and less often cystine, all of which are unfermentable. The proteid is removed by coagulation, and the others are detected by the polariscope after complete fermentation. The fermentable levulose is detected in a special manner (see below), and the dextrorotatory milk-sugar differs from dextrose in its not fermenting readily. By using a delicate instrument and with sufficient practice very exact results can be obtained by this method. The value of this procedure consists in the rapidity with which the determination can be made. In using instruments specially constructed for clinical purposes the accuracy is less than with the less expensive fermentation test. Under such circumstances, and as the estimation by means of polarization can be performed with exactitude only by specially trained chemists, it is hardly worth while to give this method in detail, and the reader is referred to handbooks for hints in the use of the apparatus.

Levulose. Levogyrate urines containing sugar have been observed by several observers, although the nature of the sugar was not well known to the earlier observers. In recent years several positively authentic cases of levulosuria have been described, and also cases of diabetes have been found where levulose exists in the urine besides dextrose.

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[1] Roberts, Edinburgh Med. Journ., 1861, and The Lancet, **1**, 1862; Worm-Müller, Pflüger's Arch., **33** and **37**; Budde, *ibid.*, **40**, and Zeitschr. f. physiol. Chem , **13** See also Huppert-Neubauer, 10. Aufl., and Lohnstein, Pflüger's Arch., **62**.

[2] Berlin. klin. Wochenschr., **35**, and Allg. med Central-Ztg , 1899.

[3] Münch. med. Wochenschr., 1905.

Levulose may be detected as follows: The urine is levorotatory and the levorotatory substance ferments with yeast. The urine gives the ordinary reduction tests and the ordinary phenylglucosazone. With methylphenylhydrazine it gives the characteristic levulose methylphenylosazone, and it also gives SELIWANOFF'S reaction on heating after the addition of an equal volume of hydrochloric acid and a little resorcin. With this test it must be remarked that too lengthy or too strong heating must not be applied, since other carbohydrates may also give the reaction (see page 119 and the works of ROSIN and UMBER). After heating and cooling it can be neutralized with soda and shaken out with amyl alcohol. This removes a red pigment which gives a band in the spectrum between *E* and *b* on stronger concentration; also a band in the blue at *F* (ROSIN).[1]

Laiose is a substance named by HUPPERT and found by LEO [2] in diabetic urines in certain cases, and which he considers as a sugar. It is levogyrate, amorphous, and does not taste sweet, but rather sharp and salty. Laiose has a reducing action on metallic oxides, does not ferment, and gives a non-crystalline, yellowish-brown oil with phenylhydrazine. There is no positive proof as yet that this substance is a sugar.

Lactose. The appearance of lactose in the urine of pregnant women was first shown by the observations of DE SINETY and F. HOFMEISTER, and this has been substantiated by other investigators. After the ingestion of large quantities of milk-sugar some lactose may be found in the urine (see Chapter IX on absorption). LANGSTEIN and STEINITZ have observed the passage of lactose and also of galactose [3] into the urine of nurslings with diseases of the stomach. The passage of lactose into the urine is called lactosuria.

The positive detection of this sugar in the urine is difficult, because it is, like dextrose, dextrogyrate and also gives the usual reduction tests. If urine contains a dextrogyrate, non-fermentable sugar which reduces bismuth solutions, then it is very probable that it contains lactose. It must be remarked that the fermentation test for lactose is, according to the experience of LUSK and VOIT,[4] best performed by using pure cultivated yeast (saccharomyces apiculatus). This yeast only ferments the dextrose, while it does not decompose the milk-sugar. If, according to VOIT, RUBNER'S test is performed without heating to boiling, but only to 80° C., the color becomes yellow or brown in the presence of lactose, instead of red. The most positive means for the detection of this sugar is to isolate the sugar from the urine. This may be done by the following method, suggested by F. HOFMEISTER:

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[1] Umber, Salkowski's Festschrift, Berlin, 1904; Rosin, *ibid.*, and Zeitschr. f. physiol. Chem., 38.

[2] Virchow's Arch., 107.

[3] Hofmeister, Zeitschr. f. physiol. Chem., 1, which also contains the pertinent literature. See also Lemaire, *ibid.*, 21; Langstein and Steinitz, Hofmeister's Beiträge, 7.

[4] Carl Voit, Ueber Die Glycogenbildung nach Aufnahme verschiedener Zuckeraten, Zeitschr. f. Biologie, 28.

Precipitate the urine with sugar of lead, filter, wash with water, unite the filtrate and wash-water, and precipitate with ammonia. The liquid filtered from the precipitate is again precipitated by sugar of lead and ammonia until the last filtrate is optically inactive. The several precipitates with the exception of the first, which contains no sugar, are united and washed with water. This precipitate is decomposed in the cold with sulphuretted hydrogen and filtered. The excess of sulphuretted hydrogen is driven off by a current of air; the acids set free are removed by shaking with silver oxide. Now filter, remove the soluble silver by sulphuretted hydrogen, treat with barium carbonate to unite with any free acetic acid present, and concentrate. Before the evaporated residue becomes syrupy it is treated with 90 per cent alcohol until a flocculent precipitate is formed which settles quickly. The filtrate from this when placed in a desiccator deposits crystals of lactose, which are purified by recrystallization, decolorizing with animal charcoal and boiling with 60–70 per cent alcohol.

Pentoses. SALKOWSKI and JASTROWITZ first found in the urine of persons addicted to the morphine habit a variety of sugar which was a pentose and yielded an osazone which melted at 159° C. Since this several other cases of pentosuria have been observed, and according to KÜLZ and VOGEL small amounts of pentose also occur in the urine of diabetics, as also in the urine of dogs with pancreatic or phlorhizin diabetes.[1]

The pentose isolated by NEUBERG from the urine in chronic pentosuria was *i*-arabinose. In alimentary pentosuria the *l*-arabinose of the plant food may be found in the urine. The appearance of pentoses in the urine after eating fruits and fruit-juices has been repeatedly observed by BLUMENTHAL and also by v. JAKSCH.[2]

A urine containing pentose reduces bismuth as well as copper solutions, although the reduction is not so rapid, but appears gradually. If only pentose is present, the urine does not ferment, but in the presence of dextrose small amounts of pentose may also undergo fermentation. The preparation of the osazone serves in the detection of pentoses; this compound when pure melts at 166–168° C., but when obtained from the urine has a melting-point of 156–160° C. The phloroglucin or orcin tests can also be employed (see page 111). Of these the last is most preferable, especially as it excludes a confusion with the conjugated glucuronic acids.

The orcin test can be performed as follows: 5 c.c. of the urine is mixed with an equal volume of HCl sp. gr. 1.19, a small amount of orcin added and the whole heated to boiling. As soon as a greenish cloudiness appears, cool the mixture off and shake carefully with amyl alcohol The amyl-alcohol solution is used in the spectroscopic examination. The precipitation of a bluish-green pigment is in itself significant.

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[1] In regard to the literature, see foot-note 1, page 110. See also Blumenthal, "Die Pentosurie," Deutsche Klinik, 1902.

[2] Blumenthal, Deutsche Klinik, 1902; v. Jaksch, Centralbl. f innere Medizin, 1906.

BIAL[1] uses as reagent 30 per cent hydrochloric acid, which contains 1 gram of orcin and 25 drops of a ferric-chloride solution (62.9 per cent of the crystalline salt) in 500 c.c. of the acid. 4–5 c.c. of the reagent is heated to boiling and then a few drops (not more than 1 c.c.) of the urine is added to the hot but not boiling liquid. In the presence of pentose the liquid turns a beautiful green. The usefulness of BIAL's reagent is questioned by several experimenters. The delicacy is not very great and the possibility of confounding with other carbohydrates is not excluded.

LÉPINE and BOULUD[2] have shown the presence of *maltose* in cases of diabetes. After boiling with hydrochloric acid the specific rotation diminishes, while the reducing power increases in such urines.

Conjugated Glucuronic Acids. Certain conjugated glucuronic acids such as menthol- and turpentine-glucuronic acid may spontaneously decompose in the urine. and in this case they may readily lead to a confusion with pentoses. The urine should be always as fresh as possible for these examinations.

A confusion of the glucuronic acids which have a reducing power on copper or bismuth solutions with dextrose and levulose can be prevented by the fermentation test. They may also be distinguished from dextrose by their optical behavior. as the conjugated glucuronic acids are levogyrate. On boiling with an acid dextrorotatory glucuronic acid is produced and the levorotation is changed to dextrorotation.

The conjugated glucuronic acids, like the pentoses, give the phloroglucin-hydrochloric-acid test. On the contrary they do not give the orcin test directly, but only after cleavage with the setting free of glucuronic acid. On using BIAL's reagent no mistaking for pentoses occurs, although this statement requires further substantiation. The pentoses may also be isolated and identified by their osazones. The occurrence of conjugated glucuronic acid in the urine is shown when the urine does not give the orcin-hydrochloric-acid reaction directly, but only after boiling with the acid. A further proof is that suggested by v. ALFTHAN.[3] 500 c.c. of the urine is benzoylated and the ester obtained saponified with sodium ethylate. The free and conjugated glucuronic acid is thus obtained as sodium compounds, insoluble in alcohol, while the pentoses, if present, remain in the alcoholic filtrate. We have no sufficient experience as to the value of this method.

The surest method is that suggested by MAYER and NEUBERG,[4] which consists in precipitating the urine with basic lead acetate, decomposing the precipitate with H_2S, boiling with dilute sulphuric acid in order to split the conjugated acid, and then after neutralizing with soda prepare the characteristic bromphenylhydrazine compound of glucuronic acid (see page 123) with *p*-bromphenylhydrazine hydrochloride and sodium acetate.

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[1] Deutsch. med. Wochenschr., 1903.

[2] Compt. rend., **132**.

[3] Arch. f. exp. Path. u. Pharm., **47**.

[4] Zeitschr. f. physiol. Chem., **29**.

Inosite occurs in the urine in albuminuria and in diabetes mellitus, but only rarely and in small quantities. Inosite is also found in the urine after the excessive drinking of water. According to HOPPE-SEYLER[1] traces of inosite occur in all normal urines.

In detecting inosite the proteid is first removed from the urine. Then concentrate the urine on the water-bath to ¼ of its original volume and precipitate with sugar of lead. The filtrate is warmed and treated with basic lead acetate as long as a precipitate is formed. The precipitate formed after twenty-four hours is washed with water, suspended in water, and decomposed with sulphuretted hydrogen. A little uric acid may separate from the filtrate after a short time. The liquid is filtered, concentrated to a syrupy consistency, and treated while boiling with 3–4 vols. alcohol. The precipitate is quickly separated. After the addition of ether to the cooled filtrate, crystals separate after a time, and these are purified by decolorization and recrystallization. With these crystals perform the tests mentioned on page 459.

Acetone Bodies (acetone, acetoacetic acid, β-oxybutyric acid). These bodies, whose occurrence in the urine and formation in the organism have been the subject of numerous investigations, occur in the urine especially in diabetes mellitus, but also in many other diseases.[2] According to v. JAKSCH and others acetone is a normal urinary constituent, though it may occur only in very small amounts (0.01 gram in twenty-four hours).

In regard to the origin of these bodies it was previously considered that they were produced by an increased destruction of protein. One of the various reasons for this was the increase in the elimination of acetone and acetoacteic acid during inanition (v. JAKSCH, FR. MÜLLER[3]). This stands also in good accord with the observations that a considerable increase in the quantity of acetone and acetoacetic acid eliminated is observed in such diseases as fevers, diabetes, digestive disturbances, mental diseases with abstinence and cachexia, where the body protein is largely destroyed. The formation of acetone bodies from protein is also indicated by the fact that acetone has been obtained as an oxidation product from gelatine and protein (BLUMENTHAL and NEUBERG, ORGLER[4]). On the other hand, no parallelism exists between the acetone bodies and the nitrogen excretion in diabetics, and the fact that in man no certain relationship exists between the acetone elimination and the nitrogen and sulphur excretion seem to show that the acetone bodies are not entirely derived from the proteins. In man the excretion of acetone does not increase with the rise in the quantity of protein, and an increase in the latter above the average causes a diminution in the elimination of acetone (ROSENFELD, HIRSCHFELD, FR. VOIT[1]). At the present time the tendency is more and more to the view that the acetone bodies do not originate from the proteins but from the fats; if they are not the only source, they are at least the most important.

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[1] Handbuch d. physiol. u. pathol. chem. Analyse, 6. Aufl., 196.

[2] In regard to the extensive literature on acetone bodies the reader is referred to Huppert-Neubauer, Harn-Analyse, 10. Aufl., and v. Noorden's Lehrb. d. Pathol. des Stoffwechsels. Berlin, 1906.

[3] v. Jaksch, Ueber Acetonurie und Diaceturie. Berlin, 1885; Fr. Müller, Bericht über die Ergebnisse des an Cetti ausgeführten Hungerversuches. Berlin. klin. Wochenschr., 1887.

[4] Blumenthal and Neuberg, Deutsch. med. Wochenschr., 1901; Orgler, Hofmeister's Beiträge, 1.

It is generally accepted that in man the carbohydrates have a strong influence on the elimination of acetone bodies, namely, the exclusion of carbohydrates from the food or the diminution in their amount or their assimilation may lead to more or less increased elimination of acetone bodies. This behavior may occur in diabetes as well as in starvation and in the above-mentioned diseased conditions. With abundant supply of carbohydrates the acetone bodies are markedly diminished or may disappear entirely, and a similar retarding action has been found by SATTA[2] to be brought about by other bodies, such as glycerine, tartaric acid, lactic acid, and citric acid. The increased excretion of acetone with carbohydrate starvation occurs also in healthy individuals with a fatty diet, or on the supply of sufficient calories in other ways (alimentary acetonuria).

If we do not accept the formation of acetone bodies from proteins, then we must admit such a formation from the fats. As proof of this there are certain cases of diabetes with strong elimination of acetone bodies (β-oxybutyric acid) where the quantity of protein transformed was too small to account for the acetone bodies (MAGNUS-LEVY). The free elimination of acetone bodies in starvation may also depend upon the fact that a great part of the body fat is consumed, and in several cases a certain relationship has been found between the fat consumed and the acetone bodies eliminated. Certain investigators (GEELMUYDEN, SCHWARZ, WALDVOGEL[3]) have also observed an increase in the acetonuria on partaking of fatty food.

There is no doubt that the fats bear a certain relationship to the acetone bodies, and that they are probably in part the source of the same. It has not been proved, on the contrary, that the fats are the only or the most important source of the acetone bodies, and to all appearances we must consider the protéins equally with the fats as the source of these bodies. The researches of EMBDEN and his coworkers are of special interest in this connection. After EMBDEN and KALBERLAH showed that the liver was an acetone-forming organ EMBDEN, SALOMON and SCHMIDT [1] showed by experiments with removed livers that butyric acid, oxybutyric acid, leucine, tyrosine, and in fact all aromatic bodies (such as tyrosine, phenylalanine, phenyl-α-lactic acid and homogentisic acid) which contain a benzene nucleus which can be burnt in the body, may be transformed into acetone in the liver.

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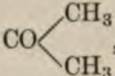
[1] Hirschfeld, Zeitschr. f. klin. Med., 28; Geelmuyden, see Maly's Jahresber., 26, and Zeitschr. f. physiol. Chem., 23 and 26; Rosenfeld, Centralbl. f. innere Med., 16; Voit, Deutsch. Arch. f. klin. Med., 66.

[2] Hofmeister's Beiträge, 6.

[3] Magnus-Levy, Arch. f. exp. Path. u. Pharm., 42; Geelmuyden, l. c., and Norsk. Magazin for Laegevidenskaben, 1900, see also Zeitschr. f. physiol. Chem. 41, Schwarz, Deutsch. Arch. f. klin. Med., 1903; Waldvogel, Centralbl. f. innere Med., 20.

In drawing conclusions as to the origin of the acetone bodies it must not be forgotten that the conditions in man are distinctly different from those in carnivora (GEELMUYDEN, FR. VOIT). In dogs the elimination of acetone bodies is not increased in starvation, but is reduced; it is augmented with increased quantities of meat, runs parallel with the nitrogen excretion, and is not diminished by carbohydrates (FR. VOIT).

Acetone, C_3H_6O, dimethylketone=  $CO\begin{smallmatrix}CH_3\\CH_3\end{smallmatrix}$, occurs, as above stated, in very small amounts in normal urine. In diabetes it may give a pomaceous or fruit odor to the urine as well as to the expired air.

Irrespective of the alimentary acetonuria derived from the food, there occurs an increased elimination of acetone, as above stated, in many diseases, as also after nervous lesions, certain intoxications, and after administration of phlorhizin or extirpation of the pancreas (v. MERING and MINKOWSKI, AZÉMAR [2]).

Acetone is a thin, water-clear liquid, boiling at 56.3° C. and possessing a pleasant odor of fruit. It is lighter than water, with which it mixes in all proportions, also with alcohol and ether. The most important reactions for acetone are the following.

LIEBEN'S *Iodoform Test.* When a watery solution of acetone is treated with alkali and then with some iodo-potassium-iodide solution and gently warmed a yellow precipitate of iodoform is formed, which is known by its odor and by the appearance of the crystals (six-sided plates or stars) under the microscope. This reaction is very delicate, but it is not characteristic of acetone. GUNNING'S *modification of the iodoform test* consists in using an alcoholic solution of iodine and ammonia instead of the iodine dissolved in potassium iodide and alkali hydrate. In this case, besides iodoform, a black precipitate of iodide of nitrogen is formed, but this gradually disappears on standing, leaving the iodoform visible. This modification has the advantage that it does not give any iodoform with alcohol or aldehyde. On the other hand, it is not quite so delicate, but still it detects 0.01 milligram of acetone in 1 c.c.

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[1] Hofmeister's Beiträge, 8.

[2] Azémar, "Acétonurie expérimentale." Travaux de physiologie, 1898 (laboratoire de M. le professeur E. Hédon, Montpellier).

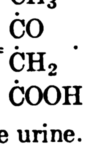
Reynolds's *mercuric-oxide test* is based on the power of acetone to dissolve freshly precipitated HgO. A mercuric-chloride solution is precipitated by alcoholic caustic potash. To this add the liquid to be tested, shake well, and filter. In the presence of acetone the filtrate contains mercury, which may be detected by ammonium sulphide. This test has about the same delicacy as Gunning's test. Aldehydes also dissolve appreciable quantities of mercuric oxide.

Legal's *Sodium Nitroprusside Test.* If an acetone solution is treated with a few drops of a freshly prepared sodium-nitroprusside solution and then with caustic-potash or soda solution, the liquid is colored ruby-red. Creatinine gives the same color; but if the mixture is saturated with acetic acid, the color becomes carmine or purplish red in the presence of acetone, but yellow and then gradually green and blue in the presence of creatinine. With this test paracresol responds with a reddish-yellow color, which becomes light pink when acidified with acetic acid and cannot be mistaken for acetone. If ammonia is employed instead of the caustic alkali (Le Nobel), the reaction takes place with acetone but not with aldehyde.

Penzoldt's *indigo test* depends on the fact that orthonitrobenzaldehyde in alkaline solution with acetone yields indigo. A warm saturated and then cooled solution of the aldehyde is treated with the liquid to be tested for acetone and next with caustic soda. In the presence of acetone the liquid first becomes yellow, then green, and lastly indigo separates; and this may be dissolved with a blue color by shaking with chloroform. 1.6 milligrams acetone can be detected by this test.

Bêla v. Bitto's[1] reaction is based on the fact than on adding a solution of metadinitrobenzene made alkaline with caustic potash to acetone, a violet-red color is produced which becomes cherry-red on acidifying with an organic acid or metaphosphoric acid. Aldehyde gives a similar violet-red color which becomes yellowish red on acidification. Creatinine does not give this reaction. Frommer[2] has suggested the following method for detecting acetone: Treat 10 c.c. of the urine with 1 gram potassium hydrate and add 10–12 drops of an alkaline solution of salicyl-aldehyde. On warming a purple-red coloration is obtained in the presence of acetone.

Acetoacetic acid, $C_4H_6O_3$, acetylacetic acid, diacetic acid =



$$\begin{array}{c} CH_3 \\ \dot{C}O \\ \dot{C}H_2 \\ \dot{C}OOH \end{array}$$

This acid has not been observed as a physiological constituent of the urine. It occurs in the urine chiefly under the same conditions as acetone. Like acetone the acetoacetic acid occurs often in children, especially in high fevers, acute exanthema, etc. Diacetic acid decomposes readily into acetone. According to ARAKI[1] it is probably produced as an intermediate product in the oxidation of β-oxybutyric acid in the organism. The three bodies appearing in the urine, acetone, acetoacetic acid, and β-oxybutyric acid, stand in close relationship to each other.

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[1] Annal. d. Chem u. Pharm., 269.

[2] Berlin. klin. Wochenschr. 1905.

This acid is a colorless, strongly acid liquid which mixes with water, alcohol, and ether in all proportions. On heating to boiling with water. and especially with acids, this acid decomposes into carbon dioxide and acetone, and therefore gives the above-mentioned reactions for acetone. It differs from acetone in that it gives a violet-red or brownish-red color with a dilute ferric-chloride solution. For the detection of this acid we make use of the following reactions which may be applied directly to the urine.

GERHARDT'S *Reaction.* Treat 10–15 c.c. of the urine with ferric-chloride solution until it fails to give a precipitate, filter, and add some more ferric chloride. In the presence of acetoacetic acid a wine-red color is obtained. The color becomes paler at the room temperature within twenty-four hours, but more quickly on boiling (differing from salicylic acid, phenol, sulphocyanides). A portion of the urine slightly acidified and boiled does not give this reaction on account of the decomposition of the acetoacetic acid.

ARNOLD *and* LIPLIAWSKY'S *Reaction.* 6 c.c. of a solution containing 1 gram of *p*-aminoacetophenone and 2 c.c. of concentrated hydrochloric acid in 100 c.c. of water are mixed with 3 c.c. of a 1 per cent potassium-nitrite solution and then treated with an equal volume of urine. A few drops of concentrated ammonia are now added and violently shaken. A brick-red coloration is obtained. Then take 10 drops to 2 c.c. of this mixture (according to the quantity of acetoacetic acid in the urine), add 15–20 c.c. HCl of sp. gr. 1.19, 3 c.c. of chloroform, and 2–4 drops of ferric-chloride solution and mix without shaking. In the presence of acetoacetic acid the chloroform is colored violet or blue (otherwise only yellowish or faintly red). This reaction is more delicate than the preceding test and reacts with 0.04 p. m. acetoacetic acid. Large amounts of acetone (but not the quantity occurring in urines) give this reaction according to ALLARD.[2]

BONDI and SCHWARZ'S[3] *Reaction.* 5 c.c. of the urine is titrated drop. by drop with iodine-potassium iodide solution until the color is orange-red Then warm gently and when the orange-red color has disappeared add the iodine solution again until the color remains permanent on warming. Then boil, when the irritating vapors of iodo-acetone will attack the eyes. Acetone does not give this reaction.

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[1] Zeitschr. f. physiol. Chem., 18.

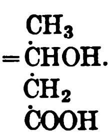
[2] Arnold, Wien. klin. Wochenschr., 1899, and Centralbl. f. innere Med., 1900; Lipliawsky, Deutsch. med. Wochenschr., 1901; Allard, Berl. klin. Wochenschr., 1901.

[3] Wien. kiln. Wochenschr., 1906.

Detection of Acetone and Acetoacetic Acid in the Urine. Before testing for acetone test for acetoacetic acid; as this acid gradually decomposes on allowing the urine to stand, the specimen must be as fresh as possible. In the presence of acetoacetic acid the urine gives the above-mentioned tests. In testing for acetone in the presence of acetoacetic acid make the urine slightly alkaline and shake in a separatory funnel with ether free from alcohol and acetone. Remove the ether and shake it with water, which takes up the acetone, and test for acetone in the watery solution.

In the absence of acetoacetic acid the acetone may be tested for directly in the urine; this may be done by PENZOLDT's test. This test, which is only approximate, is of value only when the urine contains a considerable amount of acetone. For a more accurate test we distill at least 250 c.c. of the urine faintly acidified with sulphuric acid, care being taken to have a good condensation. Most of the acetone is contained in the first 10–20 c.c. of the distillate. A better result may be obtained by distilling a large quantity of urine until about $\frac{1}{10}$ has been distilled off, acidify the distillate with hydrochloric acid, redistill and repeat this several times, collecting the first portion of each distillation. The final distillate is used for the above reactions.[1] SALKOWSKI and BORCHARDT have called attention to the fact that in the distillation of an acidified urine containing sugar for the detection or estimation of acetone a substance giving iodoform can be formed from the sugar if the distillation is carried too far. According to BORCHARDT[2] the urine must therefore first be diluted with water or the concentration prevented by the addition of water dropwise during distillation.

The quantitative estimation of acetone in the urine is done by converting it first into iodoform. The urine is acidified with acetic acid (according to HUPPERT, 1–2 c.c. 50 per cent acetic acid for every 100 c.c. urine) and distilled. The quantity of acetone in the distillate is best determined according to MESSINGER and HUPPERT's method by determining volumetrically the quantity of iodine used in the formation of iodoform. In regard to this method and its execution the reader is referred to HUPPERT-NEUBAUER.[3]



β-Oxybutyric Acid, $C_4H_8O_3 = \begin{array}{l} CH_3 \\ \dot{C}HOH \\ \dot{C}H_2 \\ \dot{C}OOH \end{array}$. The occurence of this acid in the urine was first positively shown by MINKOWSKI, KÜLZ, and STADELMANN.[4] It occurs especially in severe cases of diabetes, when it may form the largest portion of the acetone bodies (Magnus-Levy, Geelmuyden). It has also been observed in scarlet fever, measles, in scurvy, and in diseases of the brain with abstinence. It seems to be always accompanied with acetoacetic acid.

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[1] See also Salkowski, Pflüger's Arch., **56**.

[2] Hofmeister's Beiträge, 8.

[3] Harnanalyse, 760, and also Geelmuyden, Zeitschr. f. anal. Chem., **35**, and Vaubel, Chem. Centralbl., 1905, 1, 1617.

[4] Minkowski, Arch. f. exp. Path. u. Pharm., 18 and **19**; Stadelmann, *ibid.*, **17**; Külz, Zeitschr. f. Biologie, **20** and **23**.

The β-oxybutyric acid ordinarily forms an odorless syrup, but may also be obtained as crystals. It is readily soluble in water, alcohol, and ether. It is levorotatory; $(\alpha)_D = -24.12°$ for solutions of 1–11 per cent and has a disturbing action upon the determination of sugar by means of the polariscope. It is not precipitated by basic lead acetate or by ammoniacal lead acetate, neither does it ferment. On boiling with water, especially in the presence of a mineral acid, this acid decomposes into *α-crotonic acid*, which melts at 71–72° C., and water: $CH_3.CH(OH).CH_2.COOH = H_2O + CH_3.CH{:}CH.COOH$. It yields acetone on oxidation with a chromic-acid mixture.

Detection of β-Oxybutyric Acid in the Urine. If a urine is still levogyrate after fermentation with yeast, the presence of oxybutyric acid is probable. A further test may be made, according to Kulz, by evaporating the fermented urine to a syrup and, after the addition of an equal volume of concentrated sulphuric acid, distilling directly without cooling. α-crotonic acid is produced which distills over, and, after collecting in a test-tube, crystals which melt at +72° C. separate on cooling. If no crystals are obtained, shake the distillate with ether, evaporate, and test the melting-point of the residue which has been washed with water. According to Minkowski the acid may be isolated as a silver salt.[1]

The Quantitative Estimation may be performed as follows, according to Bergell:[2] 100–300 c.c. of the sugar-free urine or fermented urine is made slightly alkaline with sodium carbonate and concentrated to a syrup. This, on cooling, is rubbed with syrupy phosphoric acid (keeping it cool), anhydrous copper sulphate (20–30 grams), and fine sand, and the dry mass thoroughly extracted with anhydrous ether in an extraction apparatus. The residue after the evaporation of the ether is dissolved in water and decolorized, if necessary, with animal charcoal, and the quantity of the acid calculated from the polarization. Other methods have been suggested by Darmstädter, Boekelman and Bouma, and Magnus-Levy.[3]

Ehrlich's[4] *Urine Test.* Mix 250 c.c. of a solution which contains 50 c.c. HCl and 1 gram of sulphanilic acid in one liter with 5 c.c. of a ½ per cent solution of sodium nitrite (which produces very little of the active body, sulphodiazobenzene). In performing this test treat the urine with an equal volume of this mixture and then supersaturate with ammonia. Normal urine will become yellow thereby, or orange after the addition of ammonia (aromatic oxyacids may sometimes after a certain time give red azo bodies which color the upper layer of the phosphate sediment). In pathological urines there sometimes occurs (and this is the characteristic diazo reaction) a primary yellow coloration, with a very marked secondary red coloration on the addition of ammonia, and the froth is also tinged with red. The upper layer of the sediment becomes greenish. The body which gives this reaction is unknown, but it occurs especially in the urine of typhoid patients (EHRLICH). Opinions differ in regard to the significance of this reaction. The fact that the antoxyproteic acid gives this reaction as above stated (page 612) is of interest.

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[1] Arch. f. exp. Path. u. Pharm., **18**, 35; Zeitschr. f. anal. Chem., **24**, 153.

[2] Zeitschr. f. physiol. Chem., **33**

[3] Darmstadter, *ibid.*, **37**; Boekelmann and Bouma, see Maly's Jahresber., **31**; Magnus-Levy, Arch. f. exp. Path. u. Pharm. 45.

[4] Ehrlich, Zeitschr. f. klin. Med., **5**. See also Clemens, Deutsch. Arch. f. klin. Med., **63** (literature).

Another urine test suggested by EHRLICH consists in adding a hydrochloric acid containing 2 per cent dimethylaminobenzaldehyde to the urine; normal urines are colored faintly red, while certain pathological urines become cherry-red. The cause of this reaction is not sufficiently known; according to NEUBAUER [1] it appears to be connected with the urobilinogen.

ROSENBACH's urine test, which consists in adding nitric acid drop by drop to the boiling-hot urine and obtaining a claret-red coloration and a bluish-red foam on shaking, depends upon the formation of indigo substances, especially indigo red.[2]

Fat in the Urine. The elimination of a urine which in appearance and richness in fat resembles chyle is called *chyluria.* It habitually contains a proteid and often fibrin. Chyluria occurs mostly in the inhabitants of the tropics. *Lipuria,* or the elimination of fat with the urine, may appear in apparently healthy persons, sometimes with and sometimes without albuminuria, in pregnancy, and also in certain diseases, as in diabetes, poisoning with phosphorus, and fatty degeneration of the kidneys.

Fat is usually detected by the microscope. It may also be dissolved with ether, and may invariably be detected by evaporating the urine to dryness and extracting the residue with ether.

Cholesterin is also sometimes found in the urine in chyluria and in a few other cases.

Amino-acids. Leucine and tyrosine have been repeatedly found by the older methods in urine, especially in acute yellow atrophy of the liver, in acute phosphorus-poisoning, and in severe cases of typhoid and smallpox. Since the use of β-naphthalene sulphochloride has been used in the detection of amino-acids these bodies have not only been repeatedly found in normal urine (glycocoll, see page 614,) but also in pathological urines. Besides an increased amount of glycocoll in certain cases of gout (ALEX. IGNATOWSKI) and the finding of tyrosine and leucine in cystinuria (ABDERHALDEN and SCHITTENHELM) and in certain other cases, ABDERHALDEN and BARKER [3] have also found phenylalanine (besides glycocoll, tyrosine, and leucine) in the urine in dogs after phosphorus poisoning.

Cystine (see page 92). BAUMANN and GOLDMANN [4] claim that a substance similar to cystine occurs in very small amounts in normal urine. This substance occurs in large quantities in the urine of dogs after poisoning with phosphorus. Cystine itself is only found with positiveness, and even then very rarely, in urinary calculi and in pathological urines, from which it may separate as a sediment. Cystinuria occurs oftener in men than in women. BAUMANN and V. UDRÁNSZKY found in urine in cystinuria the two diamines, *cadaverine* (pentamethylendiamine) and *putrescine* (tetramethylendiamine), which are produced in the putrefaction of proteins. These two diamines were also found in the contents of the intestine in cystinuria, while under normal conditions they are not present. HAMMARSTEN therefore considers that perhaps some connection exists between the formation of diamines in the intestine, by the peculiar putrefaction in cystinuria, and cystinuria itself. This is less probable and cystinuria is, as generally admitted, rather an anomaly in the protein metabolism where the cystine for unknown reasons is not destroyed as ordinarily, although sometimes those having cystinuria can quantitatively destroy the cystine introduced. Cases of cystinuria may occur with or without the occurrence of diamines in the urine, and only rarely are the diamines found in the urine as well as in the feces, which perhaps depends upon the fact, as found by CAMMRIDGE and GARROD[1] in one case, that the diamines occur only from time to time in the feces. The properties and reactions of cystine have been given on pages 92 and 93.

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[1] See Pröscher, Zeitschr. f. physiol. Chem., **31**, and Clemens, Deutsch. Arch. f. klin. Med., **71**; Neubauer, Centralbl. f. Physiol **19**, 145.

[2] See Rosin, Virchow's Arch., **123**.

[3] Ignatowski, Zeitschr. f. physiol. Chem. **42**; Abderhalden and Schittenhelm, *ibid.* **45**; Abderhalden and Barker, *ibid.* **42**.

[4] Baumann, Zeitschr. f. physiol. Chem., **8**. In regard to the literature on cystine see Brenzinger, *ibid.*, **16**; Baumann and Goldmann, *ibid.*, **12**; Baumann and v.

Cystine is easily prepared from cystine calculi by dissolving them in alkali carbonate, precipitating the solution with acetic acid, and redissolving the precipitate in ammonia. The cystine crystallizes on the spontaneous evaporation of the ammonia. The cystine dissolved in the urine is detected, in the absence of proteid and sulphuretted hydrogen, by boiling with alkali and testing with a lead salt or sodium nitroprusside. To isolate cystine from the urine, acidify the urine strongly with acetic acid. The precipitate containing cystine is collected after twenty-four hours and digested with hydrochloric acid, which dissolves the cystine and calcium oxalate, leaving the uric acid undissolved. Filter, supersaturate the filtrate with ammonium carbonate, and treat the precipitate with ammonia, which dissolves the cystine and leaves the calcium oxalate. Filter again and precipitate with acetic acid. The precipitated cystine is identified by the microscope and the above-mentioned reactions. Cystine as a sediment is identified by the microscope. It must be purified by dissolving in ammonia and precipitating with acetic acid and then further tested. Traces of dissolved cystine may be detected by the production of benzoyl-cystine, according to BAUMANN and GOLDMAN.

Udránszky, *ibid.*, **13**; Stadthagen and Brieger, Berlin. klin. Wochenschr., 1889; Cammidge and Garrod, Journ. of Path. and Bacteriol. 1900 (literature on diamines in the urine and fæces).

[1] Journ. of Path. and Bacteriol., 1900.

VII. Urinary Sediments and Calculi.

Urinary sediment is the more or less abundant deposit which is found in the urine after standing. This deposit may consist partly of organized and partly of non-organized constituents. The first, consisting of cells of various kinds, yeast-fungi, bacteria, spermatozoa, casts, etc., must be investigated by means of the microscope, and the following only applies to the non-organized deposits.

As previously mentioned (page 543), the urine of healthy individuals may sometimes, even on voiding, be cloudy on account of the phosphates present, or become so after a little while because of the separation of urates. As a rule, urine just voided is clear, and after cooling shows only a faint cloud (nubecula) which consists of urine mucoid, a few epithelium-cells mucous corpuscles, and urate particles. If an acid urine is allowed to stand, it will gradually change; it becomes darker and deposits a sediment consisting of uric acid or urates, and sometimes also calcium-oxalate crystals, in which yeast-fungi and bacteria are often to be seen. This change, which the earlier investigators called "ACID FERMENTATION OF THE URINE," is generally considered as an exchange of the dihydrogen alkali phosphates with the urates of the urine. Monohydrogen phosphates besides acid urates or free uric acid or a mixture of both, according to conditions,[1] are hereby formed.

Sooner or later, sometimes only after several weeks, the reaction of the original acid urine changes and becomes neutral or alkaline. The urine has now passed into the "ALKALINE FERMENTATION," which consists in the decomposition of the urea into carbon dioxide and ammonia by means of lower organisms, micrococcus ureæ, bacterium ureæ, and other bacteria. MUSCULUS[2] has isolated an enzyme from the micrococcus ureæ which decomposes urea, is soluble in water and is called *urease.* During the alkaline fermentation volatile fatty acids, especially acetic acid, may be produced, chiefly by the fermentation of the carbohydrates of the urine (SALKOWSKI[3]). A fermentation by which nitric acid is reduced to nitrous acid, and another where sulphuretted hydrogen is produced, may sometimes occur.

When the alkaline fermentation has advanced only so far as to render the reaction neutral, there often occur in the sediment fragments of uric-acid crystals, sometimes covered with prismatic crystals of alkali urate; dark-colored spheres of ammonium urate, crystals of calcium oxalate, and sometimes crystallized calcium phosphate are also found. Crystals of ammonium-magnesium phosphate (triple phosphate) and spherical ammonium urate are specially characteristic of alkaline fermentation. The urine in alkaline fermentation becomes paler and is often covered with a fine membrane which contains amorphous calcium phosphate and glistening crystals of triple phosphate and numerous micro-organisms.

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[1] See Huppert-Neubauer, 10. Aufl., and A. Ritter, Zeitschr. f. Biologie, **35**.

[2] Musculus, Pflüger's Arch., **12**.

[3] Salkowski, Zeitschr. f. physiol. Chem., **13**.

Non-organized Sediments.

Uric Acid. This acid occurs in acid urines as colored crystals which are identified partly by their form and partly by their property of giving the murexid test. On warming the urine they are not dissolved. On the addition of caustic alkali to the sediment the crystals dissolve, and when a drop of this solution is placed on a microscope-slide and treated with a drop of hydrochloric acid small crystals of uric acid are obtained which can be easily seen under the microscope.

Acid Urates. These occur only in the sediment of acid or neutral urines. They are amorphous, clay-yellow, brick-red, rose-colored, or brownish red. They differ from other sediments in that they dissolve on warming the urine. They give the murexid test, and small microscopic crystals of uric acid separate on the addition of hydrochloric acid. Crystalline alkali urates occur very rarely in the urine, and as a rule only in such as have become neutral but not alkaline by alkaline fermentation. The crystals are somewhat similar to those of neutral calcium phosphate; they are not dissolved by acetic acid, however, but give a cloudiness therewith due to small crystals of uric acid.

Ammonium urate may indeed occur as a sediment in a neutral urine which at first was strongly acid and has become neutralized by the alkaline fermentation, but it is only characteristic of ammoniacal urines. This sediment consists of yellow or brownish rounded spheres which are often covered with thorny-shaped prisms and, because of this, are rather large and resemble the thorn-apple. It reacts to the murexid test. It is dissolved by alkalies with the development of ammonia, and crystals of uric acid separate on the addition of hydrochloric acid to this solution.

Calcium oxalate occurs in the sediment generally as small, shining, strongly refractive quadratic octahedra, which on microscopical examination remind one of a letter-envelope. The crystals can only be mistaken for small, not fully developed crystals of ammonium-magnesium phosphate. They differ from these by their insolubility in acetic acid. The oxalate may also occur as flat, oval, or nearly circular disks with central cavities which from the side appear like an hour-glass. Calcium oxalate may occur as a sediment in an acid as well as in a neutral or alkaline urine.

The quantity of calcium oxalate separated from the urine as sediment depends not only upon the amount of this salt present but also upon the acidity of the urine. The solvent for the oxalate in the urine seems to be the diacid alkali phosphate, and the greater the quantity of this salt in the urine the greater the quantity of oxalate in solution. When, as previously mentioned (page 677), the simple-acid phosphate is formed from the diacid phosphate, on allowing the urine to stand, a corresponding part of the oxalate may be separated as sediment.

Calcium carbonate occurs in considerable quantities as sediment in the urine of herbivora. It occurs in but small quantities as a sediment in human urine, and in fact only in alkaline urines. It either has almost the same appearance as amorphous calcium oxalate or it occurs as somewhat larger spheres with concentric bands. It dissolves in acetic acid with the generation of gas, which differentiates it from calcium oxalate. It is not yellow or brown like ammonium urate, and does not give the murexid test.

Calcium Phosphate. The CALCIUM TRIPHOSPHATE, $Ca_3(PO_4)_2$, which occurs only in alkaline urines, is always amorphous and occurs partly as a colorless, very fine powder and partly as a membrane consisting of very fine granules. It differs from the amorphous urates in that it is colorless, dissolves in acetic acid, but remains undissolved on warming the urine. CALCIUM DIPHOSPHATE, $CaHPO_4+2H_2O$, occurs in neutral or only in very faintly acid urine. It is found sometimes as a thin film covering the urine and sometimes as a sediment. In crystallizing, the crystals may be single, or they may cross one another, or they may be arranged in groups of colorless, wedge-shaped crystals whose wide end is sharply defined. These crystals differ from crystalline alkali urates in that they dissolve without a residue in dilute acids and do not give the murexid test

Calcium sulphate occurs very rarely as a sediment in strongly acid urine. It appears as long, thin, colorless needles, or generally as plates grouped together.

Ammonium-magnesium phosphate, TRIPLE PHOSPHATE, may separate from an amphoteric urine in the presence of a sufficient quantity of ammonium salts, but it is generally characteristic of a urine which is ammoniacal through alkaline fermentation. The crystals are so large that they may be seen with the unaided eye as colorless glistening particles in the sediment, on the walls of the vessel, and in the film on the surface of the urine. This salt forms large prismatic crystals of the rhombic system (coffin-shaped) which are easily soluble in acetic acid. Amorphous *magnesium triphosphate,* $Mg_3(PO_4)_2$, occurs with calcium triphosphate in urines rendered alkaline by a fixed alkali. Crystalline magnesium phosphate, $Mg_3(PO_4)_2+22H_2O$, has been observed in a few cases in human urine (also in horse's urine) as strongly refractive, long rhombic plates.

Kyestein is the film which appears after a little while on the surface of the urine. This coating, which was formerly considered as characteristic of urine in pregnancy, contains various elements, such as fungi, vibriones, epithelium-cells, etc. It often contains earthy phosphates and triple-phosphate crystals.

As more rare sediments we find *cystine, tyrosine, hippuric acid, xanthine, hæmatoidine.* In alkaline urine blue crystals of *indigo* may also occur, due to a decomposition of indoxyl-glucuronic acid.

Urinary Calculi.

Besides certain pathological constituents of the urine, all those urinary constituents which occur as sediments take part in the formation of urinary calculi. EBSTEIN [1] considers the essential difference between an amorphous or crystalline sediment in the urine on one side and urinary sand or large calculi on the other to be the occurrence of an organic frame in the latter. As the sediments which appear in normal acid urine and in a urine alkaline through fermentation are diverse, so also are the urinary calculi which appear under corresponding conditions.

If the formation of a calculus and its further development take place in an undecomposed urine, it is called a PRIMARY formation. If, on the contrary, the urine has undergone alkaline fermentation and the ammonia formed thereby has given rise to a calculus formation by precipitating ammonium urate, triple phosphate, and earthy phosphates, then it is called a SECONDARY formation. Such a formation takes place, for instance, when a foreign body in the bladder produces catarrh accompanied by alkaline fermentation.

We discriminate between the nucleus or nuclei—if such can be seen—and the different layers of the calculus. The nucleus may be essentially different in different cases, for quite frequently it consists of a foreign body introduced into the bladder. The calculus may have more than one nucleus. In a tabulation made by ULTZMANN of 545 cases of vesicular calculi, the nucleus in 80.9 per cent of the cases consisted of uric acid (and urates); in 5.6 per cent, of calcium oxalate; in 8.6 per cent, of earthy phosphates; in 1.4 per cent, of cystine; and in 3.5 per cent, of some foreign body.

During the growth of a calculus it often happens that, for some reason or other, the original calculus-forming substance is covered with another layer of a different substance. A new layer of the original substance may deposit on the outside of this, and this process may be repeated. In this way a calculus consisting originally of a simple stone may be converted into a so-called compound stone with several layers of different substances. Such calculi are always formed when a primary is changed into a secondary formation. By the continued action of an alkaline urine containing pus, the primary constituents of an originally primary calculus may be partly dissolved and be replaced by phosphates. Metamorphosed urinary calculi are formed in this way.

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[1] Die Natur und Behandlung der Harnsteine. Wiesbaden, 1884.

Uric-acid calculi are very abundant. They are variable in size and form. The size of the bladder-stone varies from that of a pea or bean to that of a goose-egg. Uric-acid stones are always colored: generally they are grayish yellow, yellowish brown, or pale red-brown. The upper surface is sometimes entirely even or smooth, sometimes rough or uneven. Next to the oxalate calculus the uric-acid calculus is the hardest. The fractured surface shows regular concentric, unequally colored layers which may often be removed as shells. These calculi are formed primarily. Layers of uric acid sometimes alternate with other layers of primary formation, most frequently with layers of calcium oxalate. The simple uric-acid calculus leaves very little residue when burnt on a platinum foil. It gives the murexid test, but there is no material development of ammonia when acted on by caustic soda.

Ammonium urate calculi occur as primary calculi in new-born or nursing infants, rarely in grown persons. They often occur as a secondary formation. The primary stones are small, with a pale-yellow or dark-yellowish surface. When moist they are almost like dough; in the dry state they are earthy, easily crumbling into a pale powder. They give the murexid test and develop much ammonia with caustic soda.

Calcium-oxalate calculi are, next to uric-acid calculi, the most abundant. They are either smooth and small (HEMP-SEED CALCULI) or larger, of the size of a hen's egg, with rough, uneven surface, or their surface is covered with prongs (MULBERRY CALCULI). These calculi produce bleeding easily, and therefore they often have a dark-brown surface due to decomposed blood-coloring matters. Among the calculi occurring in man these are the hardest. They dissolve in hydrochloric acid without developing gas, but are not soluble in acetic acid. After gently heating the powder, it dissolves in acetic acid with frothing. With more intense heat it becomes alkaline, due to the production of quicklime.

Phosphate Calculi. These, which consist mainly of a mixture of the normal phosphate of the alkaline earths with triple phosphate, may be very large. They are as a rule of secondary formation and contain besides these phosphates also some ammonium urate and calcium oxalate. These calculi ordinarily consist of a mixture of three constituents — earthy phosphate, triple phosphate, and ammonium urate — surrounding a foreign body as a nucleus. Their color is variable — white, dingy white, pale yellow, sometimes violet or lilac-colored (from indigo red). The surface is always rough. Calculi consisting of triple phosphate alone are seldom found. They are ordinarily small, with granular or radiated crystalline fracture. Stones of mono-acid calcium phosphate are also seldom obtained. They are white and have beautiful crystalline texture. The phosphatic calculi do not burn up, the powder dissolves in acid without effervescence, and the solution gives the reactions for phosphoric acid and the alkaline earths. The triple-phosphate calculi generate ammonia on the addition of an alkali.

Calcium-carbonate calculi occur chiefly in herbivora. They are seldom found in man. They have mostly chalky properties, and are ordinarily white. They are completely or in great part dissolved by acids with effervescence.

Cystine calculi occur but seldom. They are of primary formation, of various sizes, sometimes as large as a hen's egg. They have a smooth or rough surface, are white or pale yellow, and have a crystalline fracture. They are not very hard and are consumed almost entirely on the platinum foil burning with a bluish flame. They give the above-mentioned reactions for cystine.

Xanthine calculi are very rarely found. They are also of primary formation. They vary from the size of a pea to that of a hen's egg. They are whitish, yellowish-brown or cinnamon-brown in color, of medium hardness, with amorphous fracture, and on rubbing appear like wax. They burn up completely when heated on a platinum foil. They give the xanthine reaction with nitric acid and alkali but this must not be mistaken for the murexid test.

Urostealith calculi have been observed only a few times. In the moist state they are soft and elastic at the temperature of the body, but in the dry state they are brittle, with an amorphous fracture and waxy appearance. They burn with a luminous flame when heated on platinum foil and generate an odor similar to resin or shellac. Such a calculus, investigated by KRUKENBERG,[1] consisted of paraffine derived from a paraffine bougie used as a sound on the patient. Perhaps the urostealith calculi observed in other cases had a similar origin, although the substances of which they consisted have not been closely studied. HORBACZEWSKI has recently analyzed a case of urostealith which, to all appearances, was formed in the bladder. This calculus contained 25 p. m. water, 8 p. m. inorganic bodies, 117 p. m. bodies insoluble in ether, and 850 p. m. organic bodies soluble in ether, among which were 515 p. m. free fatty acids, 335 p. m. fat, and traces of cholesterin. The fatty acids consisted of a mixture of stearic, palmitic, and probably myristic acids.

HORBACZEWSKI[2] has also analyzed a bladder stone which contained 958.7 p. m. *cholesterin.*

Fibrin calculi sometimes occur. They consist of more or less changed fibrin coagulum. On burning they develop an odor of burnt horn.

The *chemical investigation of urinary calculi* is of great practical importance. To make such an examination actually instructive it is necessary to investigate separately the different layers which constitute the calculus. For this purpose saw the calculus, previously wrapped in paper, with a fine saw so that the nucleus becomes accessible. Then peel off the different layers, or, if the stone is to be kept, scrape off enough of the powder from each layer for examination. This powder is then tested by heating on the platinum foil. It must not be forgotten that a calculus is never entirely burnt up, and also that it is never so free from organic matter that on heating it does not carbonize. Do not, therefore, lay too great stress on a very insignificant unburnt residue or on a very small amount of organic matter, but consider the calculus in the former case as completely burnt and in the latter as unaffected.

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[1] Chem. Untersuch. z. wissensch. Med., **2**. Cited from Maly's Jahresber., **19**, 422.

[2] Zeitschr. f. physiol. Chem., **18**.

When the powder is in great part burnt up, but a significant quantity of unburnt residue remains, then the powder in question contains as a rule urates mixed with inorganic bodies. In such cases remove the urate with boiling water and then test the filtrate for uric acid and the suspected bases. The residue is then tested according to the following *schema* of HELLER, which is well adapted to the investigation of urinary calculi. In regard to the more detailed examination the reader is referred to special works on the subject.

On heating the powder on platinum foil, it						
Does not burn	The powder when treated with HCl	Does not effervesce	The powder gently heated and treated with HCl	The powder when moistened with a little KHO	Abundant ammonia. The powder dissolves in acetic acid or HCl. This solution gives a crystalline precipitate with ammonia	Triple phosphate (mixed with unknown amount of earthy phosphate)
					No NH_3, or at least only traces of NH_3. Powder dissolves in acetic acid or HCl. This solution is precipitated by ammonia (amorphous)	Bone earth (phosphate of calcium and magnesium)
				Effervesces		Calcium oxalate
		Effervesces				Calcium carbonate
Does burn	With flame				Flame yellow, continuous. Odor of burnt feathers. Insoluble in alcohol and ether. Soluble in KHO with heat. Precipitated herefrom by acetic acid and generation of H_2S	Fibrin
					Flame yellow, pale, continuous. Odor of resin or shellac on burning. Powder soluble in alcohol and ether	Urostealith
					Flame pale blue, burns a short time. Peculiar sharp odor. The powder dissolves in ammonia, and six-sided plates separate on the spontaneous evaporation of the ammonia	Cystine
	Without flame				Does not give the murexid test. The powder dissolves in HNO_3 without effervescence. The dried yellow residue becomes orange with alkali, beautiful red on warming	Xanthine
		The powder gives the murexid test	The powder when treated with KHO gives		Strong ammonia reaction	Ammonium urate
					No noticeable ammonia reaction	Uric acid

CHAPTER XVI.

THE SKIN AND ITS SECRETIONS.

In the structure of the skin of man and vertebrates many different kinds of substances occur which have already been considered, such as the constituents of the epidermal formation, the connective and fatty tissues, the nerves, muscles, etc. Among these the different horn structures, the hair, nails, etc., whose chief constituent, keratin, has been spoken of in another chapter (Chapter II), are of special interest.

The cells of the horny structure show, in proportion to their age, a different resistance to chemical reagents, especially fixed alkalies. The younger the horn-cell the less resistance it has to the action of alkalies; with advancing age the resistance becomes greater, and the cell-membranes of many horn-formations are nearly insoluble in caustic alkalies. Keratin occurs in the horn structure mixed with other bodies, from which it is isolated with difficulty. Among these bodies the mineral constituents in many cases occupy a prominent place because of their quantity. Hair leaves on burning 5–70 p. m. ash, which may contain in 1000 parts 230 parts alkali sulphates, 140 parts calcium sulphate, 100 parts iron oxide, and even 400 parts silicic acid. Dark hair on burning seems generally, although not always, to yield more iron oxide than blond. The nails are rich in calcium phosphate, and the feathers rich in silicic acid, which Drechsel[1] claims exists in part in organic combination as an ester.

According to Gautier and Bertrand[2] arsenic also occurs in the epidermal formations. The arsenic is, according to Gautier, of importance in the formation and growth of the same, and on the other hand these structures, hair, nails, and epidermis-cells, are of great importance for the excretion of arsenic.

The skin of invertebrates has been the subject, in a few cases, of chemical investigation, and in these animals various substances have been found, of which a few, though little studied, are worth discussing. Among these bodies *tunicin*, which is found especially in the mantle of the tunicata, and the widely diffused *chitin*, found in the cuticle-formation of invertebrates, are of interest.

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[1] Centralbl. f. Physiol., **11**, 361.

[2] Gautier, Compt. rend., **129**, **130**, **131**; Bertrand, *ibid.*, **134**.

Tunicin. Cellulose seems, according to the investigations of Ambronn, to occur rather extensively in the animal kingdom in the arthropoda and the mollusks. It has been known for a long time as the mantle of the *tunicata*, and this animal cellulose was called tunicin by Berthelot. According to the investigations of Winterstein there does not seem to exist any marked difference between tunicin and ordinary vegetable cellulose. On boiling with dilute acid tunicin yields dextrose, as shown first by Franchimont[1] and later confirmed by Winterstein.

Chitin is not found in vertebrates. In invertebrates chitin is alleged to occur in several classes of animals; but it can be positively asserted that true, typical chitin is found only in articulated animals, in which it forms the chief organic constituent of the shell, etc. According to Krawkow[2] chitin of the shell, etc., does not seem to occur free, but in combination with another substance, probably a proteid-like body. Chitin also occurs, according to Gilson and Winterstein,[3] in certain fungi.

According to Sundvik the formula of chitin is probably $C_{60}H_{100}N_8O_{38} + n(H_2O)$, where n may vary between 1 and 4. According to Araki it has on the contrary the composition $C_{18}H_{30}N_2O_{12}$. According to Krawkow the chitins of different origin show different behavior with iodine, and he therefore concludes that there must exist quite a group of chitins, which seem to be amine derivatives of different carbohydrates, such as dextrose, glycogen, dextrins, etc. According to Zander[4] only two chitins exist, one of which turns violet with iodine and zinc chloride, and the other brown.

Chitin is decomposed on boiling with mineral acids and yields, as shown by Ledderhose, *glucosamine* and *acetic acid*. Schmiedeberg, therefore, considers chitin as a probable acetyl acetic-acid combination of glucosamine. Fränkel and Kelly,[5] on the contrary, consider chitin as of a more complicated composition. The most characteristic cleavage product obtained by them was a chitosamine acetylized at the nitrogen atom, $C_6H_{12}O_5N.COCH_3$, and a second product, acetyldichitosamine, $C_{14}H_{26}O_{10}N_2$, which, according to Araki, has the same composition as chitosan (see below), but is essentially different in many regards.

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[1] Ambronn, Maly's Jahresber., **20**; Berthelot, Annal. de Chim, et Phys., **56**, Compt. rend., **47**; Winterstein, Zeitschr. f. physiol. Chem., **18**; Franchimont, Ber. d. deutsch chem. Gesellsch., **12**.

[2] Zeitschr. f. Biologie, **29**.

[3] Gilson, Compt. rend., **120**: Winterstein, Ber. d. deutsch. chem. Gesellsch., **27** and **28**.

[4] Sundvik, Zeitschr. f. physiol. Chem., **5**; Araki. *ibid.* **20**; Zander, Pflüger's Arch., **66**.

[5] Ledderhose, Zeitschr. f. physiol. Chem., **2** and **4**; Schmiedeberg, Arch. f. exp. Path. u. Pharm., **28**; Fränkel and Kelly, Monatshefte f. Chem., **23**.

According to **Hoppe-Seyler** and **Araki**,[1] on heating chitin with alkali and a little water to 180° C. a cleavage takes place with the splitting off of acetic acid, and the formation of a new substance, *chitosan*, whose formula according to **Araki** is $C_{14}H_{26}N_2O_{10}$, but according to v. **Fürth** and **Russo**[2] more likely a multiple of $C_{13}H_{26}N_2O_{14}$. On heating with acetic anhydride chitosan is converted into a chitin-like substance which is not identical with chitin. Chitosan is insoluble in water and alkali, but dissolves in dilute acids. It splits into acetic acid and glucosamine by the action of hydrochloric acid. According to v. **Fürth** and **Russo** on acid cleavage it yields 25 per cent acetic acid and 60 per cent glucosamine. One nitrogen atom corresponds closely to 1 molecule acetic acid and $\frac{3}{4}$ molecule glucosamine. All the glucosamine complexes present in the chitosan molecule seem to be acetylized. According to **Krawkow** the various chitins behave differently with iodine or with sulphuric acid and iodine, in that some are colored reddish brown, blue, or violet, while others are not colored at all.

In a dry state chitin forms a white, brittle mass retaining the form of the original tissue. It is insoluble in boiling water, alcohol, ether, acetic acid, dilute mineral acids, and dilute alkalies. It is soluble in concentrated acids. It is dissolved without decomposing in cold concentrated hydrochloric acid, but is decomposed by boiling hydrochloric acid. When chitin is dissolved in concentrated sulphuric acid and the solution dropped into boiling water and then boiled, a substance is obtained (glucosamine, chitosamine) which reduces copper suboxide in alkaline solutions.

Chitin may be easily prepared from the wings of insects or from the shells of the lobster or the crab, the last-mentioned having first been extracted by an acid so as to remove the lime salts. The wings or shells are boiled with caustic alkali until they are white, afterward washed with water, then with dilute acid and water, and lastly extracted with alcohol and ether. If chitin so prepared is dissolved in cold, concentrated sulphuric acid and diluted with cold water, then pure chitin separates out, having been set free from the combination with the other bodies (**Krawkow**).

Hyalin is the chief organic constituent of the walls of hydatid cysts. From a chemical point of view it stands close to chitin, or between it and protein. In old and more transparent sacs it is tolerably free from mineral bodies, but in younger sacs it contains a great quantity (16 per cent) of lime salts (carbonate, phosphate, and sulphate).

According to **Lücke**[3] its composition is:

	C	H	N	O
From old cysts	45.3	6.5	5.2	43.0
From young cysts	44.1	6.7	4.5	44.7

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[1] Araki. l. c.; v. Fürth and Russo, Hofmeister; Beitrage 8.

[3] Virchow's Arch., 19.

It differs from keratin on the one hand and from proteids on the other by the absence of sulphur, also by its yielding, when boiled with dilute sulphuric acid, a variety of sugar in large quantities (50 per cent), which is reducing, fermentable, and dextrogyrate. It differs from chitin by the property of being gradually dissolved by caustic potash or soda, or by dilute acids; also by its solubility on heating with water to 150° C.

The coloring matters of the skin and horn-formations are of different kinds, but have not been much studied. Those occurring in the stratum Malpighii of the skin, especially of the negro, and the black or brown pigment occurring in the hair, belong to the group of those substances which have received the name *melanins*.

Melanins. This group includes several different varieties of amorphous black or brown pigments which are insoluble in water, alcohol, ether, chloroform, and dilute acids, and which occur in the skin, hair, epithelium-cells of the retina, in sepia, in certain pathological formations, and in the blood and urine in disease. Of these pigments there are a few, such as the melanin of the eye, SCHMIEDEBERG'S *sarcomelanin*, and that from the melanotic sarcomata of horses, the *hippomelanin* (NENCKI, SIEBER, and BERDEZ), which are soluble with difficulty in alkalies, while others, such as the coloring matter of certain pathological swellings in man, the *phymatorhusin* (NENCKI and BERDEZ), are readily soluble in alkalies. The humus-like products, called *melanoidic acids* by SCHMIEDEBERG, obtained on boiling proteins with mineral acids, are rather easily soluble in alkalies.

Among the melanins there are a few, for example the choroid pigment, which are free from sulphur (LANDOLT and others); others, on the contrary, as sarcomelanin and the pigment of the hair and of horse-hair, are rather rich in sulphur (2–4 per cent), while the phymatorhusin found in certain swellings and in the urine (NENCKI and BERDEZ, K. MÖRNER) is very rich in sulphur (8–10 per cent). Whether any of these pigments, especially the phymatorhusin, contains any iron or not is an important though disputed point, for it leads to the question whether these pigments are formed from the blood-coloring matters. According to NENCKI and BERDEZ the pigment, phymatorhusin, isolated by them from a melanotic sarcoma did not contain any iron, and according to them is not a derivative of hæmoglobin. K. MÖRNER and later also BRANDL and L. PFEIFFER found, on the contrary, that this pigment did contain iron, and they consider it as a derivative of the blood-pigments. The *sarcomelanin* (from a sarcomatous liver) analyzed by SCHMIEDEBERG contained 2.7 per cent iron, which was in organic combination in part and could not be completely removed by dilute hydrochloric acid. The *sarcomelanic acid* prepared by SCHMIEDEBERG by the action of alkali on this melanin contained 1.07 per cent iron. The sarcomelanin investigated by ZDAREK and v.

Zeynek also contained 0.4 per cent iron. Recently Wolff[1] has prepared two pigments from a melanotic liver of which one was no doubt modified. The other, which was soluble in a soda solution, contained 2.51 per cent sulphur and 2.63 per cent iron, which was in great part split off by 20 per cent hydrochloric acid. From another liver he obtained on the contrary a melanin free from iron with 1.67 per cent sulphur. From this melanin he obtained, by treatment with bromine, a hydro-aromatic body which was related to xyliton (a condensation product of acetone).[2]

The difficulties which attend the isolation and purification of the melanins have not been overcome in certain cases, while in others it is questionable whether the final product obtained has not another composition from the original coloring matter, owing to the energetic chemical processes resorted to in its purification. Under these circumstances and as no doubt we have a large number of melanins having different composition, it seems that a tabulation of the analyses of the different melanin preparations can only be of secondary importance.

The one or more pigments of the human hair have a low percentage of nitrogen, 8.5 per cent (Sieber), and a variable but considerable amount of sulphur, 2.71–4.10 per cent. The great quantity of iron oxide which remains on incinerating hair does not seem to belong to the pigments. The pigment of the negro's skin and hair was found entirely free from iron by Abel and Davis.[3] The pigment prepared by Spiegler from the hair of animals also contained no iron.

So little is known about the structural products of the melanins or melanoids that it is impossible to give the origin of these bodies. As undoubtedly there are several distinct melanins, their origin must also be distinct. The ferruginous melanins should be considered as originating from the blood-pigments until further research proves otherwise. Most melanins — and this is also true for the melanoids produced from proteins on cleavage with acids (Samuely) — yield indol or skatol and a pyrrol substance, and we must therefore admit with Samuely[4] that the different chromogen groups contained in the protein molecule, which readily yield aromatic and specially heterocyclic nuclei, which condense with the withdrawal of water and absorption of oxygen, produce dark colored products the mixture of which forms the melanoids.

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[1] Zdarek and v. Zeynek, Zeitschr. f. physiol. Chem., **36**; Wolff, Hofmeister Beiträge **5**. The literature on the melanins may be found in Schmiedeberg, "Elementarformeln einiger Eiweisskörper, etc." Arch. f. exp. Path. u. Pharm., **39**; also in Kobert, Wiener Klinik, **27** (1901), and Spiegler, Hofmeister's Beiträge, **4**.

[2] The summary of the extensive literature on melanotic pigments may be found in O. v. Fürth, Centralbl. f. allgem. Path. u. Pathol. Anat. **15**, 1904.

[3] Journ. of Expt. Med. **1**, 361.

[4] Hofmeister's Beiträge, **2**.

It has also been found that by the action of tyrosinases upon tyrosine dark products similar to melanin are formed, and these, like the animal melanins, yield substances smelling like skatol on fusion with alkali. Such a direct pigment formation caused by the presence of tyrosinase has also been observed by GESSARD in the maceration of the skin of frogs and toads, and certain investigators, such as GESSARD, v. FÜRTH and SCHNEIDER,[1] are therefore of the opinion that tyrosine is the mother-substance of the melanins.

In addition to the coloring matters of the human skin it is in place here to treat of the pigments found in the skin or epidermal formation of animals.

The beautiful color of the feathers of many birds depends in certain cases on purely physical causes (interference-phenomena), but in other cases on coloring matters of various kinds. Such a coloring matter is the amorphous reddish-violet *turacin*, which contains 7 per cent copper and whose spectrum is very similar to that of oxyhæmoglobin. It must be remarked that according to LAIDLAW[2] turacin or at least a pigment with the same properties can be obtained on boiling hæmatoporphyrin in dilute ammonia with ammoniacal copper solution. KRUKENBERG[3] found a large number of coloring matters in birds' feathers, namely, *zooerythrin, zoofulvin, turacoverdin, zoorubin, psittacofulvin*, and others which cannot be enumerated here.

Tetronerythrin, so named by WURM, is a red amorphous pigment which is soluble in alcohol and ether, and which occurs in the red warty spots over the eyes of the heathcock and the grouse, and which is very widely spread among the invertebrates (HALLIBURTON, DE MEREJKOWSKI, MACMUNN). Besides tetronerythrin MACMUNN found in the shells of crabs and lobsters a blue coloring matter *cyanocrystallin*, which turns red with acids and by boiling water. *Hæmatoporphyrin*, according to MACMUNN,[4] also occurs in the integuments of certain of the lower animals.

In certain butterflies (the pieridinæ) the white pigment of the wings consists, as shown by HOPKINS,[5] of uric acid, and the yellow pigment of a uric-acid derivative, *lepidotic acid*, which yields a purple substance, *lepidoporphyrin*, on warming with dilute sulphuric acid. The yellow and red pigment of the Vanessa are, according to LINDEN,[6] of an entirely different kind. In this case we are dealing with a compound between proteid and a pigment which is allied to bilirubin or urobilin, i.e., a compound similar to hæmoglobin.

In addition to the coloring matters thus far mentioned a few others found in certain animals (though not in the skin) will be spoken of.

Carminic acid, or the red pigment of the cochineal, gives on oxidation, according to LIEBERMANN and VOSWINCKEL,[7] *cochenillic acid*, $C_{10}H_8O_7$, and *coccinic acid*.

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[1] Gessard, Compt. rend. **136**, and Compt. rend., soc. biol. **57**; v. Fürth and Schneider, Hofmeister's Beiträge, **1**.

[2] Journ. of Physiol. **31**.

[3] Vergleichende physiol. Studien, Abth. **5**, and (2. Reihe) Abth. **1**, 151, Abth. **2**, 1, and Abth. **3**, 128.

[4] Wurm, cited from Maly's Jahresber., **1**; Halliburton, Journ. of Physiol., **6**; Merejkowski, Compt. rend., **93**; MacMunn, Proc. Roy. Soc., 1883, and Journ. of Physiol., **7**.

[5] Phil. Trans., **186**.

[6] Pflüger's Arch., **98**.

[7] Ber. d. deutsch. chem. Gesellsch., **30**.

$C_9H_8O_5$, the first being the tri-carboxylic acid, and the other the di-carboxylic acid of *m*-cresol. The beautiful purple solution of ammonium carminate has two absorption-bands between *D* and *E* which are similar to those of oxyhæmoglobin. These bands lie nearer to *E* and closer together and are less sharply defined. *Purple* is the evaporated residue from the purple-violet secretion, caused by the action of the sunlight, from the so-called "purple gland" of the mantle of certain species of *murex* and *purpura*. Its chemical nature has not been investigated.

Among the remaining coloring matters found in invertebrates may be mentioned *blue stentorin, actiniochrom, bonellin, polyperythrin, pentacrinin, antedonin, crustaceorubin, janthinin,* and *chlorophyll.*

Sebum when freshly secreted is an oily semi-fluid mass which solidifies on the upper surface of the skin, forming a greasy coating. Sebum is according to RÖHMANN and LINSER a mixture of the secretion of the sebaceous glands and of the constituents of the epidermis. HOPPE-SEYLER has found in the sebum a body similar to casein besides albumin and fat. According to RÖHMANN and LINSER true fat occurs only to a very slight extent. On saponification the sebum gives an oil, *dermolein,* which combines readily with iodine, and another body, *dermocerin,* which melts at 64–65° and which occurs to a considerable extent in dermoid cysts and which is perhaps identical with the constituent of cysts called cetylalcohol by v. ZEYNEK. The amount of cholesterin in this secretion is small and originates essentially from the epidermoidal formation. Cholesterin is found in especially large quantities in the *vernix caseosa.* The solids of the sebum consist chiefly of fat, epithelium-cells, and protein bodies; the *vernix caseosa* is made up chiefly of fat. RÜPPEL[1] found on an average in the *vernix caseosa* 348.52 p. m. water and 138.72 p. m. ether extractives. Besides cholesterin he found also isocholesterin.

On account of the generally diffused view that the wax of the plant epidermis serves as protection for the inner parts of the fruit and plant, LIEBREICH[2] has suggested that these combinations of fatty acids with monatomic alcohols are the cause of the waxes having a greater resistance as compared with the glycerine fats. He also considers that the cholesterin fats play the rôle of a protective fat in the animal kingdom, and he has been able to detect cholesterin fat in human skin and hair, in *vernix caseosa,* whalebone, tortoise-shell, cow's horn, the feathers and beaks of several birds, the spines of the hedgehog and porcupine, the hoofs of horses, etc. He draws the following conclusion from this, namely, that the cholesterin fats always appear in combination with the keratinous substance, and that the cholesterin fat, like the wax of plants, serves as protection for the skin-surface of animals.

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[1] Hoppe-Seyler, Physiol. Chem. 760; Linser with Röhmann, Centralbl. f. Physiol. **19**, 317; see also reference in *ibid.* **18** from Deutsch. Arch. f. klin. Med., 1904; Rüppel Zeitschr. f. physiol. Chem., **21**.

[2] Virchow's Arch., **121**.

In the fatty protective substance secreted by the Psylla alni SUNDVIK[1] has found psylla-alcohol, $C_{33}H_{68}O$, which exists there as an ester in combination with psyllic acid, $C_{32}H_{65}COOH$.

Cerumen is a mixture of the secretion of the sebaceous and sweat glands of the cartilaginous part of the outer passages of the ear. It contains chiefly soaps and fat, fatty acids, cholesterin and proteid, and besides these a red substance easily soluble in alcohol and with a bitter-sweet taste.[2]

The **preputial secretion,** *smegma præputii,* contains chiefly fat, also cholesterin and ammonium soaps, which probably are produced from decomposed urine. The hippuric acid, benzoic acid, and calcium oxalate found in the smegma of the horse have probably the same origin.

We may also consider as a preputial secretion the *castoreum*, which is secreted by two peculiar glandular sacs in the prepuce of the beaver. The castoreum is a mixture of proteins, fat, resins, traces of phenol (volatile oil), and a non-nitrogenous body, *castorin*, crystallizing in four-sided needles from alcohol, insoluble in cold water, but somewhat soluble in boiling water, and whose composition is little known.

In the secretion from the anal glands of the skunk butyl mercaptan and alkyl sulphide have been found (ALDRICH, E. BECKMANN[3]).

Wool-fat, or the so-called fat-sweat of sheep, is a mixture of the secretion of the sudoriparous and sebaceous glands. There is found in the watery extract a large quantity of potassium which is combined with organic acid, volatile and non-volatile fatty acids, benzoic acid, phenol-sulphuric acid, lactic acid, malic acid, succinic acid, and others. The fat contains, among other bodies, abundant quantities of ethers of fatty acids with cholesterin and isocholesterin. DARMSTÄDTER and LIFSCHÜTZ have found other alcohols in wool-fat besides myristic acid, also two oxyfatty acids, *lanoceric acid*, $C_{30}H_{60}O_4$, and *lanopalmitic acid*, $C_{16}H_{32}O_2$. According to RÖHMANN[4] wool-fat contains a body *lanocerin*, which is the internal anhydride of the above-mentioned lanoceric acid. Lanocerin is obtained without saponification by repeatedly boiling lanolin with methyl alcohol, dissolving the insoluble residue in ether and precipitating with alcohol.

The secretion of the coccygeal glands of ducks and geese contains a body similar to casein, besides albumin, nuclein, lecithin, and fat, but no sugar (DE JONGE). The chief constituent is *octadecyl alcohol*, $C_{18}H_{38}O$, which represents 40–45 per cent of the ethereal extract (RÖHMANN). The fatty acids are oleic acid, small amounts of caprylic acid, palmitic acid, and stearic acid, and optical isomers of lauric and myristic acid. The fatty acids are in great part combined with the octadecylic acid, and this is probably formed by the reduction of stearic acid or oleic acid. The secretion also contains a substance related to lanocerin which RÖHMANN calls *pennacerin*. Poisonous bodies have been found in the secretion of the skin of the salamander and the toad, namely, *samandarin* (ZALESKI, FAUST) and *bufidin* (JORNARA and CASALI), *bufotalin* and the disputed bodies *bufonin* and *bufotenin*

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[1] Zeitschr. f. physiol. Chem., **17**, **25**, and **32**.

[2] See Lamois and Martz, Maly's Jahresber., **27**, 40.

[3] Aldrich, Journ. of Expt. Med., **1**; Beckmann, Maly's Jahresber., **26**, 566.

[4] Darmstädter and Lifschütz, Ber. d. d. Chem., Gesellsch. **29** and **31**; Röhmann, Hofmeisters Beiträge **5** and Centrabl. f. Physiol. **19**, 317.

(FAUST, BERTRAND and PHISALIX[1]). *Thalassin* is the crystalline body discovered by RICHET[2] which is the poisonous constituent of the feelers of the sea nettle.

The Perspiration. Of the secretions of the skin, whose quantity amounts to about $\frac{1}{64}$ of the weight of the body, a disproportionally large part consists of water. Next to the kidneys, the skin in man is the most important means for the elimination of water. As the glands of the skin and the kidneys stand near to each other in regard to their functions, they may to a certain extent act vicariously.

The circumstances which influence the secretion of perspiration are very numerous, and the quantity of sweat secreted must consequently vary considerably. The secretion differs for different parts of the skin, and it has been stated that the perspiration of the cheek, that of the palm of the hand, and that under the arm stand to each other as 100:90:45. From the unequal secretion on different parts of the body it follows that no results as to the quantity of secretion for the entire surface of the body can be calculated from the quantity secreted by a small part of the skin in a given time. In determining the total quantity a stronger secretion is as a rule produced, and as the glands can with difficulty work for a long time with the same energy, it is hardly correct to estimate the quantity of secretion per day from a strong secretion during only a short time.

The perspiration obtained for investigation is never quite pure, but contains cast-off epidermis-cells, also cells and fat-globules from the sebaceous glands. Filtered perspiration is a clear, colorless fluid with a salty taste and of different odors from different parts of the body. The physiological reaction is acid, according to most statements. Under certain conditions also an alkaline sweat may be secreted (TRÜMPY and LUCHSINGER, HEUSS). An alkaline reaction may also depend on a decomposition with the formation of ammonia. According to a few investigators the physiological reaction is alkaline, and an acid reaction depends, according to them, upon an admixture of fatty acids from the sebum. CAMERER found that the reaction of human perspiration in certain cases was acid and in others alkaline. MORIGGIA found that the sweat from herbivora was ordinarily alkaline, while that from carnivora was generally acid. According to SMITH[3] horse's sweat is strongly alkaline.

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[1] De Jonge, Zeitschr. f. physiol. Chem., **3**; Röhmann l. c.; Zaleski, Hoppe-Seyler's Méd.-chem. Untersuch., **85**; Faust, Arch. f. exp. Path. u. Pharm., **41**; Jornara and Casali, Maly's Jahresber., **3**; Faust, Arch. f. exp. Path. u. Pharm., **47** and **49**; Bertrand, Compt. rend., **135**; Bertrand and Phisalix, *ibid.*

[2] Pflüger's Arch., **108**.

[3] Trümpy and Luchsinger, Pflüger's Arch., **18**; Heuss, Maly's Jahresber., **22**; Camerer, Zeitschr. f. Biologie, **41**; Moriggia, Moleschott's Untersuch. zur Naturlehre, **11**; Smith, Journ. of Physiol., **11**. In regard to the older literature on perspiration, see Hermann's Handbuch, **5**, Thl. 1, 421 and 543.

The specific gravity of human perspiration varies between 1.001 and 1.010. It contains 977.4–995.6 p. m., average about 982 p. m. water. The solids are 4.4–22.6 p. m. The molecular concentration is also very variable and the freezing-point depression depends essentially upon the content of NaCl. Ardin-Delteil found $\Delta = -0.08 - 0.46°$, average $-0.237°$. Brieger and Disselhorst[1] found with perspiration containing 2.9, 7.07 and 13.5 p. m. NaCl, that the Δ was equal to $-0.322°$, $-0.608°$ and $-1.002°$, respectively. The organic bodies are *neutral fats, cholesterin, volatile fatty acids*, traces of *protein* (according to Leclerc and Smith always in horses, and according to Gaube regularly in man, while Leube[2] claims only sometimes after hot baths, in Bright's disease, and after the use of pilocarpin), also *creatinine* (Capranica), *aromatic oxyacids, ethereal-sulphuric acids* of *phenol* and *skatoxyl* (Kast[3]), sometimes also of indoxyl, and lastly *urea*. The quantity of urea has been determined by Argutinsky. In two steam-bath experiments, in which in the course of $\frac{1}{2}$ and $\frac{3}{4}$ hour respectively he obtained 225 and 330 c. c. of perspiration, he found 1.61 and 1.24 p. m. urea. Of the total nitrogen of the perspiration in these two experiments 68.5 per cent and 74.9 per cent respectively belong to the urea. From Argutinsky's experiments, and also from those of Cramer,[4] it follows that of the total nitrogen a portion not to be disregarded is eliminated by the perspiration. This portion was indeed 12 per cent in an experiment of Cramer at high temperature and powerful muscular activity. Cramer has also found ammonia in the perspiration. In uræmia, and in anuria in cholera, urea may be secreted in such quantities by the sweat-glands that crystals deposit upon the skin. The mineral bodies consist chiefly of sodium chloride with some potassium chloride, alkali sulphate, and phosphate. The relative quantities of these in perspiration differ materially from the quantities in the urine (Favre,[5] Kast). The relationship, according to Kast, is as follows:

	Chlorine	:	Phosphate	:	Sulphate
In perspiration.	1	:	0.0015	:	0.009
In urine.	1	:	0.1320	:	0.397

Kast found that the proportion of ethereal-sulphuric acid to the sulphate-sulphuric acid in perspiration was 1:12. After the administration of aromatic substances the ethereal-sulphuric acid does not increase to the same extent in the perspiration as in the urine (see Chapter XV).

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[1] Ardin-Delteil, Maly's Jahresber., **30**; Brieger and Disselhorst, Deutsch. med. Wochenschr., **29**.

[2] Leclerc, Compt. rend., **107**; Gaube, Maly's Jahresber., **22**; Leube, Virchow's Arch., **48** and **50**, and Arch. f. klin. Med., **7**.

[3] Capranica, Maly's Jahresber., **12**; Kast, Zeitschr. f. physiol. Chem., **11**.

[4] Argutinsky, Pflüger's Arch., **46**; Cramer, Arch. f. Hygiene, **10**.

[5] Compt. rend., **35**. and Arch. génér. de Med. (5), **2**.

Sugar may pass into the perspiration in diabetes, but the passage of the bile-coloring matters has not been positively shown in this secretion. *Benzoic acid, succinic acid, tartaric acid, iodine, arsenic, mercuric chloride,* and *quinine* pass into the perspiration. *Uric acid* has also been found in the perspiration in gout and *cystine* in cystinuria.

Chromhidrosis is the name given to the secretion of colored perspiration. Sometimes perspiration has been observed to be colored blue by indigo (BIZIO), by pyocyanin, or by ferro-phosphate (KOLLMANN[1]). True blood-sweat, in which blood-corpuscles exude from the opening of the glands, has also been observed.

The *exchange of gas through the skin* in man is of very little importance compared with the exchange of gas by the lungs. The absorption of oxygen by the skin, which was first shown by REGNAULT and REISET, is very small, and according to ZUELZER amounts under the most favorable circumstances to $\frac{1}{150}$ of the oxygen absorbed by the lungs. The quantity of carbon dioxide eliminated by the skin increases with the rise of temperature (AUBERT, RÖHRIG, FUBINI and RONCHI, BARRATT[2]). It is also greater in light than in darkness. It is greater during digestion than when fasting, and greater after a vegetable than after an animal diet (FUBINI and RONCHI). The quantity calculated by various investigators for the entire skin surface in twenty-four hours varies between 2.23 and 32.8 grams.[3] In a horse, ZUNTZ with LEHMANN and HAGEMANN,[4] found for twenty-four hours an elimination of carbon dioxide by the skin and intestine which amounted to nearly 3 per cent of the total respiration. Less than four-fifths of this carbon dioxide came from the skin respiration. According to the same investigators the skin respiration equals 2½ per cent of the simultaneous lung respiration.

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[1] Bizio, Wien. Sitzungsber., **39**; Kollmann, cited from v. Gorup-Besanez's Lehrbuch, 4. Aufl., 555.

[2] Zuelzer, Zeitschr. f. klin., Med., **53**; Aubert, Pflüger's Arch., **6**; Röhrig, Deutsch. Klin., 1872, 209; Fubini and Ronchi, Moleschott's Untersuch. z. Naturlehre, **12**; Barratt, Journ. of Physiol., **21**.

[3] See Hoppe-Seyler, Physiol. Chem., 580.

[4] Arch. f. (Anat., u.). Physiol., 1894, and Maly's Jahresber., **24**.

CHAPTER XVII.

CHEMISTRY OF RESPIRATION.

DURING life a constant exchange of gases takes place between the animal body and the surrounding medium. Oxygen is inspired and carbon dioxide expired. This exchange of gases, which is called respiration, is brought about in man and vertebrates by the nutritive fluids, blood and lymph, which circulate in the body and which are in constant communication with the outer medium on one side and the tissue-elements on the other. Such an exchange of gaseous constituents may take place wherever the anatomical conditions offer no obstacle, and in man it may go on in the intestinal tract, through the skin, and in the lungs. As compared with the exchange of gas in the lungs, the exchange already mentioned, which occurs in the intestine and through the skin, is very insignificant. For this reason we will discuss in this chapter only the exchange of gas between the blood and the air of the lungs on one side and the blood and lymph and the tissues on the other. The first is often designated as external respiration, and the other, internal respiration.

I. The Gases of the Blood.

Since the pioneer investigations of MAGNUS and LOTHAR MEYER the gases of the blood have formed the subject of repeated careful investigations by prominent experimenters, among whom must be mentioned first C. LUDWIG and his pupils and E. PFLÜGER and his school. By these investigations not only has science been enriched by a mass of facts, but also the methods themselves have been made more perfect and accurate. In regard to these methods, as also in regard to the laws of the absorption of gases by liquids, dissociation, and related questions, the reader is referred to text-books on physiology, on physics, and on gasometric analysis.

The gases occurring in blood under physiological conditions are *oxygen*, *carbon dioxide* and *nitrogen*, and traces of argon, hydrogen, hydrocarbons and carbon monoxide. The nitrogen is found only in very small quantities, on an average 1.2 vols. per cent. The quantity is here, as in all following experiments, calculated for 0° C. and 760 mm. pressure. The nitrogen seems to be simply absorbed by the blood, at least in great part.

It appears, like argon, to play no direct part in the processes of life, and its quantity varies but slightly in the blood of different blood-vessels.

The oxygen and carbon dioxide behave otherwise, as their quantities have significant variations, not only in the blood from different blood-vessels, but also because many conditions, such as a difference in the rapidity of circulation, a different temperature, rest and activity, cause a change. In regard to the gases they contain the greatest difference is observable between the blood of the arteries and that of the veins.

The *quantity of oxygen* in the arterial blood of dogs is on an average 22 vols. per cent (PFLÜGER). In human blood SETSCHENOW found about the same quantity, namely, 21.6 vols. per cent. Lower figures have been found for rabbit's and bird's blood, respectively 13.2 per cent and 10–15 per cent (WALTER JOLYET). Venous blood in different vascular regions has very variable quantities of oxygen. By summarizing a great number of analyses by different experimenters ZUNTZ has calculated that the venous blood of the right side of the heart contains on an average 7.15 per cent less oxygen than the arterial blood.

The *quantity of carbon dioxide* in the arterial blood (of dogs) is about 40 vols. per cent (LUDWIG, SETSCHENOW, PFLÜGER, P. BERT, BOHR and HENRIQUES and others), or a little above. SETSCHENOW found 40.3 vols. per cent in human arterial blood. The quantity of carbon dioxide in venous blood varies still more (LUDWIG, PFLÜGER, and their pupils, P. BERT, MATHIEU and URBAIN, and others). According to the calculations of ZUNTZ, the venous blood of the right side of the heart contains about 8.2 per cent more carbon dioxide than the arterial. The average amount may be put down as 50 vols. per cent. HOLMGREN found in blood after asphyxiation even 69.21 vols. per cent carbon dioxide.[1]

Oxygen is absorbed only to a small extent by the plasma, which only absorbs 0.65 per cent oxygen. The greater part or nearly all of the oxygen is loosely combined with the hæmoglobin. The quantity of oxygen which is contained in the blood of the dog corresponds closely to the quantity which from the activity of the hæmoglobin we should expect to combine with oxygen, and from the quantity of hæmoglobin contained therein. It is difficult to ascertain how far the circulating arterial blood is saturated with oxygen, as immediately after bleeding a loss of oxygen always takes place. Still it seems to be unquestionable that it is not quite completely saturated with oxygen in life.

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[1] All the figures given above may be found in Zuntz's "Die Gase des Blutes" in Hermann's Handbuch d. Physiol., 4, Thl. 2, 33–43, which also contains detailed statements and the pertinent literature, and Bohr in Nagel's Handbuch der Physiologie des Menschen, Bd. 1, Hefte 1, 1905.

The carbon dioxide of the blood occurs in part, and indeed, according to the investigations of ALEX. SCHMIDT,[1] ZUNTZ,[2] and L. FREDERICQ,[3] to the extent of at least one-third in the blood-corpuscles, also in part, and in fact the greatest part, in the plasma or serum. According to BOHR[4] a pressure of about 30 mm. may be considered as the average pressure of the carbon dioxide in the organism, and with such a pressure the quantity of physically dissolved CO_2 in 100 c.c. of the blood amounts to 2.01 c.c. As the blood with this tension takes up about 40 vols. per cent CO_2, hence about 5 per cent of the total carbon dioxide is simply dissolved. Under the assumption that the blood corpuscles make up about $\frac{1}{3}$ of the volume of the blood, of the physically dissolved CO_2, 0.59 c.c. exists with the corpuscles and 1.42 c.c. with the plasma.

As the blood corpuscles in 100 c.c. blood as above stated take up at the above pressure about 14 c.c. CO_2 only a small part of its CO_2 is physically dissolved. The chief mass of the CO_2 is loosely combined and the constituent of these cells which unites with the CO_2 seems to be the alkali combined with phosphoric acid, oxyhæmoglobin or hæmoglobin, and globulin on one side and the hæmoglobin itself on the other. That in the red blood-corpuscles alkali phosphate occurs in such quantities that it may be of importance in the combination with carbon dioxide is not to be doubted; and it must be allowed that from the diphosphate, by a greater partial pressure of the carbon dioxide, monophosphate and alkali carbonate are formed, while by a lower partial pressure of the carbon dioxide the mass action of the phosphoric acid comes again into play, so that, with the carbon dioxide becoming free, a re-formation of alkali diphosphate takes place. It is generally admitted that the blood-coloring matters, especially the oxyhæmoglobin which can expel carbon dioxide from sodium carbonate *in vacuo*, acts like acids; and as the globulins also act similarly (see below), these bodies may also occur in the blood-corpuscles as an alkali combination. The alkali of the blood-corpuscles must therefore, according to the law of mass action, be divided between the carbon dioxide, phosphoric acid, and the other constituents of the blood-corpuscles which possess acidic properties, and among these especially the blood pigments, because the globulin can hardly be of importance on account of its small quantity. By greater mass action or greater partial pressure of the carbon dioxide, bicarbonate must be formed at the expense of the diphosphates and the other alkali combinations, while at a diminished partial pressure of the same gas, with the escape of carbon dioxide, the alkali diphosphate and the other alkali combinations must be re-formed at the cost of the bicarbonate.

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[1] Ber. d. k. sächs. Gesellsch. d. Wissensch., math.-phys. Klasse, 1867.

[2] Centralbl. f. d. med. Wissensch., 1867, 529.

[3] Recherches sur la constitution du Plasma sanguin, 1878, 50, 51.

[4] In regard to the work of Bohr we will refer here and in future to Nagel's Handbuch der Physiologie des Menschen, Bd. 1, Hefte 1.

Hæmoglobin must nevertheless, as the investigations of SETSCHENOW[1] and ZUNTZ, and especially those of BOHR and TORUP,[2] have shown, be able to hold the carbon dioxide loosely combined even in the absence of alkali. BOHR has also found that the dissociation curve of the carbon-dioxide hæmoglobin corresponds essentially to the curve of the absorption of carbon dioxide, on which ground he and TORUP consider the hæmoglobin itself as of importance in the binding of the carbon dioxide of the blood, and not its alkali combinations. According to BOHR the hæmoglobin takes up the two gases, oxygen and carbon dioxide, simultaneously by the oxygen uniting with the pigment nucleus and the carbon dioxide with the protein component. But as according to the researches of ZUNTZ[3] the combination of hæmoglobin with the alkali is first split to any great extent with a carbon dioxide tension of more than 70 mm., it must be admitted that with the ordinary CO_2 pressure in the organism, the combination of the carbon dioxide in the blood corpuscles does not essentially take place through the agency of the alkali but chiefly by means of the hæmoglobin.

The chief part of the carbon dioxide of the blood is found in the blood-plasma or the blood-serum, which follows from the fact that the serum is richer in carbon dioxide than the corresponding blood itself. By experiments with the air-pump on blood-serum it has been found that the chief part of the carbon dioxide contained in the serum is given off in a vacuum, while a smaller part can be removed only after the addition of an acid. The red blood-corpuscles also act as an acid, and therefore in blood all the carbon dioxide is expelled *in vacuo*. Hence a part of the carbon dioxide is in firm chemical combination in the serum.

Absorption experiments with blood-serum have shown us further that the carbon dioxide which can be pumped out is in great part loosely chemically combined, and from this loose combination of the carbon dioxide it necessarily follows that the serum must also contain simply absorbed carbon dioxide. For the form of binding of the carbon dioxide contained in the serum or the plasma there are the three following possibilities: 1. A part of the carbon dioxide is simply absorbed; 2. Another part is in loose chemical combination; 3. A third part is in firm chemical combination.

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[1] Centralbl. f. d. med. Wissensch., 1877. See also Zuntz in Hermann's Handbuch, 76.

[2] Zuntz, l. c., 76; Bohr, Maly's Jahresber., 17; Torup, *ibid.*

[3] Centralbl. f. d. med. Wissensch., 1867.

The quantity of physically dissolved carbon dioxide in the serum cannot be higher than about 2 vols. per cent, as the quantity of carbon dioxide in the plasma corresponding to 100 c.c. of blood is given above as 1.42 c.c.

The quantity of carbon dioxide in the blood-serum which is combined as a firm chemical union depends upon the quantity of simple alkali carbonate in the serum. This amount is not known, and it cannot be determined either by the alkalinity found by titration, nor can it be calculated from the excess of alkali found in the ash, because the alkali is not only combined with carbon dioxide, but also with other bodies, especially with protein. The quantity of carbon dioxide in firm chemical combination cannot be ascertained after pumping out *in vacuo* without the addition of acid, because to all appearances certain active constituents of the serum, acting like acids, expel carbon dioxide from the simple carbonate. The quantity of carbon dioxide not expelled from dog-serum by vacuum alone without the addition of acid amounts to 4.9 to 9.3 vols. per cent, according to the determinations of PFLÜGER.[1]

From the occurrence of simple alkali carbonates in the blood-serum it naturally follows that a part of the loosely combined carbon dioxide of the serum which can be pumped out must exist as bicarbonate. The occurrence of this combination in the blood-serum has also been directly shown. In experiments with the pump, as well as in absorption experiments, the serum behaves in other ways different from a solution of bicarbonate, or carbonate of a corresponding concentration; and the behavior of the loosely combined carbon dioxide in the serum can be explained only by the occurrence of bicarbonate in the serum. By means of vacuum the serum always allows much more than one half of the carbon dioxide to be expelled, and it follows from this that in the pumping out not only may a dissociation of the bicarbonate take place, but also a conversion of the double sodium carbonate into a simple salt. As we know of no other carbon-dioxide combination besides the bicarbonate in the serum from which the carbon dioxide can be set free by simple dissociation *in vacuo*, it must be assumed that the serum contains other weak acids, in addition to the carbon dioxide, which contend with it for the alkalies, and which expel the carbon dioxide from simple carbonates *in vacuo*. The carbon dioxide which is expelled by means of the pump, and which, without regard to the quantity merely absorbed, is generally designated as "carbon dioxide in loose chemical combination," is thus only obtained in part in dissociable loose combinations; in part it originates from the simple carbonates, from which it is expelled *in vacuo* by other weak acids.

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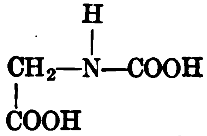
[1] E. Pflüger, Ueber die Kohlensäure des Blutes, Bonn, 1864, 11. Cited from Zuntz in Hermann's Handbuch, 65.

These weak acids are thought to be in part phosphoric acid and in part globulins. The importance of the alkali phosphates for the carbon-dioxide combination has been shown by the investigations of FERNET; but the quantity of these salts in the serum is, at least in certain kinds of blood, for example in ox-serum, so small that it can hardly be of importance. In regard to the globulins SETSCHENOW is of the opinion that they do not act as acids themselves, but form a combination with carbon dioxide, producing carboglobulinic acid, which unites with the alkali. According to SERTOLI,[1] whose views have found a supporter in TORUP, the globulins themselves are the acids which are combined with the alkali of the blood-serum. In both cases the globulins would form, directly or indirectly, that chief constituent of the plasma or of the blood-serum which, according to the law of mass action, contends with the carbon dioxide for the alkalies. By a greater partial pressure of the carbon dioxide the latter deprives the globulin alkali of a part of its alkali and bicarbonate is formed; by low partial pressure carbon dioxide is set free and it is abstracted from the bicarbonate by the globulin alkali.

The assumption that the proteins of the blood are bodies active in combining with the carbon dioxide has received some support by the investigations of SIEGFRIED[2] on the combination of carbon dioxide by amphoteric amino bodies. SIEGFRIED has found that amino acids combine with carbon dioxide, thereby being converted into carbamino-acids (glycocoll) for example, into carbamino acetic acid  $\underset{\displaystyle \text{COOH}}{\text{CH}_2}\text{—}\overset{\displaystyle \text{H}}{\text{N}}\text{—COOH}$ and that the carbon dioxide can be readily split off from these compounds. The peptones and serum proteids in the presence of calcium hydroxide may also act in the same manner as amino acids. Proteid carbamino acids are formed, and the possibility of such a binding of carbon dioxide must also be considered.

In the foregoing it has been assumed that the alkali is the most essential and important constituent of the blood-serum, as well as of the blood in general, in uniting with the carbon dioxide. The fact that the quantity of carbon dioxide in the blood greatly diminishes with a decrease in the quantity of alkali strengthens this assumption. Such a condition is found, for example, after poisoning with mineral acids. Thus WALTER found only 2–3 vols. per cent carbon dioxide in the blood of rabbits into whose stomachs hydrochloric acid had been introduced. In the comatose state of diabetes mellitus the alkali of the blood seems to be in great part saturated with acid combinations, β-oxybutyric acid (STADELMANN, MINKOWSKI), and MINKOWSKI[1] found only 3.3 vols. per cent carbon dioxide in the blood in diabetic coma.

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[1] Hoppe-Seyler, Med. chem. Untersuch.

[2] Zeitschr. f. physiol. Chem., 44 and 46.

Gases of the Lymph and Secretions.

The gases of the lymph are the same as in the blood-serum, and the lymph stands close to the blood-serum in regard to the quantity of the various gases, as well as to the kind of carbon-dioxide combination. The investigations of DAENHARDT and HENSEN[2] on the gases of human lymph are at hand, but it still remains a question whether the lymph investigated was quite normal. The gases of normal dog-lymph were first investigated by HAMMARSTEN.[3] These gases contained traces of oxygen and consisted of 37.4–53.1 per cent CO_2 and 1.6 per cent N at 0° C. and 760 mm. Hg pressure. About one half of the carbon dioxide was in firm chemical combination. The quantity was greater than in the serum from arterial blood, but smaller than from venous blood.

The remarkable observation of BUCHNER that the lymph collected after asphyxiation is poorer in carbon dioxide than that of the breathing animal is explained by, ZUNTZ[4] by the formation of acid in the tissues, and especially in the lymphatic glands, immediately after death, and this acid decomposes the alkali carbonates of the lymph in part.

The secretions with the exception of the saliva, in which PFLÜGER and KÜLZ found respectively 0.6 per cent and 1 per cent oxygen, are nearly free from oxygen. The quantity of nitrogen is the same as in blood, and the chief mass of the gases consists of carbon dioxide. The quantity of this gas is chiefly dependent upon the reaction, i.e., upon the quantity of alkali. This follows from the analyses of PFLÜGER. He found 19 per cent carbon dioxide removable by the air-pump and 54 per cent firmly combined carbon dioxide in a strongly alkaline bile, but, on the contrary, 6.6 per cent carbon dioxide removable by the air-pump and 0.8 per cent firmly combined carbon dioxide in a neutral bile. Alkaline saliva is also very rich in carbon dioxide. As average for two analyses made by PFLÜGER of the submaxillary saliva of a dog we have 27.5 per cent carbon dioxide removable by the air-pump and 47.4 per cent chemically combined carbon dioxide, making a total of 74.9 per cent. KÜLZ[5] found a maximum of 65.78 per cent carbon dioxide for the parotid saliva, of which 3.31 per cent was removable by the air-pump and 62.47 per cent was firmly combined. From these and other statements on the quantity of carbon dioxide removable by the air-pump and chemically combined in the alkaline secretions it follows that bodies occur in them, although not in appreciable quantities, which are analogous to the proteid bodies of the blood-serum and which act like weak acids.

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[1] Walter, Arch. f. exp. Path. u. Pharm., **7**; Stadelmann, *ibid.*, **17**; Minkowski, Mittheil a. d. med. Klink in Königsberg, 1888.

[2] Virchow's Arch., **37**.

[3] Ber. d. k. sächs. Gesellsch. d. Wissensch., math.-phys. Klasse, **23**.

[4] Buchner, Arbeiten aus der physiol. Anstalt zu Leipzig, 1876; Zuntz, l. c., **85**.

[5] Pflüger, Pflüger's Arch., **1** and **2**; Külz, Zeitschr. f. Biologie, **23**. It seems as if Külz's results were not calculated at 760 millimeters Hg, but rather at 1 meter.

The acid or at any rate non-alkaline secretions, urine and milk, contain, on the contrary, considerably less carbon dioxide, which is nearly all removable by the air-pump, and a part seems to be loosely combined with the sodium phosphate. The figures found by **Pflüger** for the total quantity of carbon dioxide in milk and urine are 10 and 18.1–19.7 per cent respectively.

Ewald[1] has made investigations on the quantity of gas in pathological transudates. He found only traces, or at least only very insignificant quantities of oxgyen in these fluids. The quantity of nitrogen was about the same as in blood; that of carbon dioxide was greater than in the lymph (of dogs), and in certain cases even greater than in the blood after asphyxiation (dog's blood). The tension of the carbon dioxide was greater than in venous blood. In exudates the quantity of carbon dioxide, especially that firmly combined, increases with the age of the fluid, while, on the contrary, the total quantity of carbon dioxide, and especially the quantity firmly combined, decreases with the quantity of pus-corpuscles.

II. The Exchange of Gas between the Blood on the One Hand and Pulmonary Air and the Tissues on the other.

In the introduction (Chapter I, p. 3) it was stated that we are to-day of the opinion, derived especially from the researches of **Pflüger** and his pupils, that the oxidations of the animal body do not take place in the fluids and juices, but are connected with the form-elements and tissues. It is nevertheless true that oxidations take place in the blood, although only to a slight extent; but these oxidations depend, it seems, upon the form-elements of the blood, hence it does not contradict the above statement that the oxidations occur exclusively in the cells and chiefly in the tissues.

The gaseous exchange in the tissues, which has been designated internal respiration, consists chiefly in that the oxygen passes from the blood in the capillaries to the tissues, while the great bulk of the carbon dioxide of the tissues originates therein and passes into the blood of the capillaries. The exchange of gas in the lungs, which is called external respiration, consists, as is seen by a comparison of the inspired and expired air, in the blood taking oxygen from the air in the lungs and giving off carbon dioxide.

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[1] C. A. Ewald, Arch. f. (Anat. u.) Physiol., 1873 and 1876.

This does not exclude the fact that in the lungs, as in every other tissue, an internal respiration takes place, namely, a combustion with a consumption of oxygen and formation of carbon dioxide. According to BOHR and HENRIQUES[1] the lungs take a very variable but always an important part in the total metabolism. This part which on an average is 33 per cent but may even rise above 60 per cent of the total metabolism depends according to these experimenters upon the fact that the intermediary metabolic products formed in the tissue are burnt in the lungs. It is also in part represented by secretory work of the lungs.

What kind of processes take part in this double exchange of gas? Is the gaseous exchange simply the result of an unequal tension of the blood on one side and the air in the lungs or tissues on the other? Do the gases pass from a place of higher pressure to one of a lower, according to the laws of diffusion, or are other forces and processes active?

These questions are closely related to that of the tension of the oxygen and carbon dioxide in the blood and in the air of the lungs and tissues.

Oxygen occurs in the blood in a disproportionately large part as oxyhæmoglobin, and the law of the dissociation of oxyhæmoglobin is of fundamental importance in the study of the tension of the oxygen in the blood.

Attempts have been made to prove this law by investigations on a pure solution of hæmoglobin and HÜFNER[2] has made very careful and important determinations on such solutions. Recent investigations of BOHR[3] and his pupils, as well as of LOEWY and ZUNTZ,[4] have shown that the conditions in the blood are different from a pure hæmoglobin solution, which, in part, may be due to a change in the hæmoglobin brought about in its preparation. A hæmoglobin solution combines firmer with oxygen than the blood, and the dissociation tension of the oxygen is greater in blood than in a hæmoglobin solution. If we graphically represent the influence of the oxygen pressure upon the power of the blood to take up oxygen by representing the oxygen tension as abscissa and the quantity of oxygen taken up as ordinate then the hæmoglobin solution shows a somewhat flatter oxygen tension curve than the blood.

The oxygen tension may be variable, as LOEWY[5] has shown, with different individuals and, as BOHR, HASSELBALCH, and KROGH[6] have found, that besides this the CO_2 present also influences the oxygen taken up, in that as the carbon dioxide tension (also within physiological limits) increases the oxygen taken up diminishes. The laws of oxygen absorption must be determined by determinations upon blood itself at the same time observing the temperature and the carbon dioxide tension. A series of determinations made by Krogh [1] upon horse's blood at 38° and a constant carbon dioxide tension will be given below. In calculating the results in column 5 the quantity of oxygen chemically combined at 150 mm. oxygen pressure is equal to 100.

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[1] Centralbl. f. Physiol. **6** and Maly's Jahresber, **27**.

[2] Arch. f. (Anat. u.) Physiol., 1890 and 1894.

[3] See Nagel's Handbuch and Krogh, Skand., Arch. f. Physiol., **16**.

[4] Arch. f. (Anat. u.) Physiol., 1904.

[5] *Ibid.*

[6] Centralbl. f. Physiol. **17** and Skand., Arch. f. Physiol, **16**.

	In 100 cc. Blood		Oxygen taken up	
Oxygen Tension in m.m.	Chemically combined Oxygen	Oxygen dissolved in plasma	Per cent chemically combined	Dissolved in 100 cc. plasma
10	6.0	0.020	30.0	0.030
20	12.9	0.041	64.7	0.061
30	16.3	0.061	81.6	0.091
40	18.1	0.081	90.4	0.121
50	19.1	0.101	95.4	0.152
60	19.5	0.121	97.6	0.182
70	19.8	0.141	98.8	0.212
80	19.9	0.162	99.5	0.243
90	19.95	0.182	99.8	0.273
150	20.00	0.303	100.0	0.455

From the above table we see that even with an oxygen tension which only amounts to one half of the oxygen pressure in the air that hæmoglobin in greatest part is saturated with oxygen. The dissociation is hence at 70–80 mm. pressure only slightly more than with a pressure of 150 mm. and indeed even with as low a pressure as 40–30 mm. still 90–80 per cent of the entire quantity of oxygen taken up chemically at 150 mm. is combined with the hæmoglobin.

From these and other observations it follows that the oxygen partial pressure may sink to one half of that existing in the atmospheric air without markedly influencing the oxygen content of the blood. This coincides also with the experience of Fränkel and Geppert [2] on the action of low air pressures upon the oxygen content of the blood of dogs. With an air pressure of 410 mm. Hg they found that the oxygen content of arterial blood was normal. With an air pressure of 378–365 mm. it was slightly diminished and only on reducing the pressure to 300 mm. was a mentionable decrease observed. A. Loewy [3] has found that the lowest oxygen pressure of the alveolar air when the exchange of material can go on normally both qualitatively and quantitatively, is equal to 30 mm. Hg.

It may be concluded from the large quantity of oxygen or oxyhæmoglobin in the arterial blood that the tension of the oxygen in the arterial blood must be relatively higher. From the investigations of several experimenters, such as P. **Bert**, **Herter**, and **Hüfner**,[1] who experimented partly on living animals and partly with hæmoglobin solutions, we may assume the tension of the oxygen in arterial blood at the temperature of the body to be equal to a partial oxygen pressure of 75–80 mm. Hg.

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[1] Skand. Arch, f. Physiol., **16**.

[2] Über die Wirknugen der verdrünnten Luft auf. den. Organismus. Berlin, 1883.

[3] A. Loewy, Untersuch., über die Respiration und Zirculation etc., Berlin 1895; also Centralbl. f. Physiol., **13**, 449 and Arch. f. (Anat. u.) Physiol, 1900.

According to **Bohr**[2] the facts are otherwise, and he has obtained remarkably higher results for the oxygen tension in arterial blood.

He experimented on dogs allowing the blood, whose coagulation had been prevented by the injection of peptone solution or infusion of the leech, to flow from one bisected carotid to the other, or from the femoral artery to the femoral vein, through an apparatus called by him an hæmataërometer. The apparatus, which is a modification of **Ludwig**'s rheometer (*stromuhr*), allowed, according to **Bohr**, of a complete interchange between the gases of the blood circulating through the apparatus and a quantity of gas whose composition was known at the beginning of the experiment and enclosed in the apparatus. The mixture of gases was analyzed after an equalization of the gases by diffusion. In this way the tension of the oxygen and carbon dioxide in the circulating arterial blood was determined. During the experiment the composition of the inspired and expired air was also determined, the number of inspirations noted, and the extent of respiratory exchange of gas measured. To be able to make a comparison between the gas tension in the blood and in an expired air whose composition was closer to the unknown composition of the alveolar air than the ordinary expired air, the composition of the expired air at the moment it passed the bifurcation of the trachea was ascertained by special calculation. The tension of the gases in this "bifurcated air" could be compared with the tension of the gases of the blood, and in such a way that the comparison took place simultaneously.

Bohr found remarkably high results for the oxygen tension in arterial blood in this series of experiments. They varied between 101 and 144 mm. Hg pressure. In eight out of nine experiments on the breathing of atmospheric air, and in four out of five experiments on breathing air containing carbon dioxide, the oxygen tension in the arterial blood was higher than the "bifurcated air." The greatest difference, where the oxygen tension was higher in the blood than in the air of the lungs, was 38 mm. Hg.

Hüfner and **Fredericq**[3] have made the objection to **Bohr**'s experiments and views that a perfect equilibrium had probably not been attained between the air in the apparatus and the gases of the blood. **Fredericq**, by new experiments, has presented strong objections to the acceptance of **Bohr**'s findings, while on the other hand **Bohr** not only defends his experiments but also finds errors in the experiments of his opponents. On the other hand **Haldane** and **Smith**'s[4] experiments making use of an entirely different principle speak for the high results found by **Bohr**.

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[1] Bert, La pression barometrique, Paris, 1878; Herter, Zeitschr. f. physiol. Chem., **3**; Hüfner, l. c.

[2] Skand. Arch. f. Physiol. **2** and Nagel's Handbuch. der Physiologie.

[3] Hüfner, Arch. f. (Anat. u.) Physiol. 1890; Frédericq, Centralbl. f. Physiol. **7** and Traveaux du laboratoire de l'institute de physiologie de Liège **5**, 1896.

[4] Haldane, Journ. of Physiol., **18**; Haldane and Smith, *ibid.*, **20**.

Haldane's method is as follows: The individual experimented upon is allowed to inspire air containing an exactly known but small quantity of carbon monoxide (0.045–0.06 per cent), until no further absorption of carbon monoxide takes place and the percentage saturation of the hæmoglobin in the arterial blood with carbon monoxide has become constant, as shown by a special titration method. This percentage saturation is dependent upon the relation between the tension of the oxygen in the blood and the tension of the carbon monoxide, as known from the composition of the inspired air. When this last and the percentage saturation with carbon monoxide and oxygen are known the oxygen tension in the blood can be easily calculated.

According to this method **Haldane** and **Smith** found still higher figures than **Bohr** for the oxygen tension in the blood, and they calculated the average tension of the oxygen in human arterial blood as 38.5 per cent of an atmosphere i.e., equal to about 293 mm. Hg.

Let us now compare the figures for the oxygen tension of the arterial blood as found by various investigators with the tension of the oxygen in the air of the lungs.

Numerous investigations as to the composition of the inspired atmospheric air as well as the expired air are at hand, and it can be said that these two kinds of air at 0° C. and a pressure of 760 mm. Hg have the following average composition in volume per cent:

	Oxygen.	Nitrogen (and argon).	Carbon Dioxide.
Atmospheric air	20.96	79.02	0.03
Expired air	16.03	79.59	4.38

The partial pressure of the oxygen of the atmospheric air corresponds at a normal barometric pressure of 760 mm. to a pressure of 160 mm. Hg. The loss of oxygen which the inspired air suffers in respiration amounts to about 4.93 per cent, while the expired air contains about one hundred times as much carbon dioxide as the inspired air.

The expired air is therefore a mixture of alveolar air with the residue of inspired air remaining in the air-passages; hence in the study of the gaseous exchange in the lungs the alveolar air must first be considered. There does not exist any direct determination of the composition of the alveolar air in man, but only approximate calculations. From the average results found by **Vierordt** in normal respiration for the carbon dioxide in the expired air, 4.63 per cent, **Zuntz**[1] has calculated the probable quantity of carbon dioxide in the alveolar air as equal to 5.44 per cent. If we start from this value, with the assumption that the quantity of nitrogen in the alveolar air does not essentially differ from the expired air, and admit that the quantity of oxygen in the alveolar air is 6 per cent less than the inspired air, it will be seen that the alveolar air contains 15 per cent oxygen. As the total pressure of the air of the lungs after deducting the aqueous tension of about 50 mm. can be calculated as about 710 mm. the partial pressure of the oxygen in man can be put at about 106 mm. and that of the carbon dioxide as about 45 mm.

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[1] See Zuntz l. c. Hermann's Handbuch 105 and 106.

There are several direct determinations of the alveolar air of dogs by PFLÜGER and his pupils WOLFFBERG and NUSSBAUM.[1] These determinations which show that the alveolar air is not much richer in carbon dioxide than the expired air have been performed by means of the so-called *lung-catheter*.

The principle of this method is as follows: By the introduction of a catheter of a special construction into a branch of a bronchus the corresponding lobe of the lung may be hermetically sealed, while in the other lobes of the same lung, and in the other lung, the ventilation remains unchanged, so that no accumulation of carbon dioxide takes place in the blood. When the cutting off lasts so long that a complete equalization between the gases of the blood and the retained air of the lungs is assumed, a sample of this air of the lungs is removed by means of the catheter and analyzed.

In the air thus obtained from the lungs WOLFFBERG and NUSSBAUM found an average of 3.6 per cent CO_2. NUSSBAUM has also determined the carbon-dioxide tension in the blood from the right heart in a case simultaneous with the catheterization of the lungs. He found nearly identical results, namely, a carbon-dioxide tension of 3.84 per cent and 3.81 per cent of an atmosphere, which also shows that complete equalization between the gases of the blood and lungs in the enclosed parts of the lungs had taken place. From these investigations it can be calculated that the quantity of oxygen in the alveolar air of dogs is about 16 per cent, which corresponds to an oxygen partial pressure of about 115 mm. Hg.

If the oxygen partial pressure in the alveolii, is put at only 106–115 mm. Hg, and compare this with about 80 mm. as found by certain investigators for the oxygen tension of the arterial blood, we find that a considerable excess remains in favor of the alveolü, and the taking up of oxygen in the lungs can simply, according to physical laws, be explained as a diffusion process. The conditions are quite different if we start with the high-tension results of BOHR, 101–144 mm. Hg, or the still higher results of HALDANE and SMITH. The oxygen tension in the blood is in many cases, according to HALDANE and SMITH, as average for various races of animals, indeed always higher than the tension in the lungs. In these cases the passage of oxygen from the lungs to the blood cannot be simply explained by a diffusion. We must therefore, with BOHR, accept a special specific activity of the lungs, and according to him a secretory activity of the lungs also exists besides diffusion.

As the views on the taking up of oxygen are disputed so also are the views on the giving up of carbon dioxide.

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[1] Wolffberg, Pflüger's Arch. 6; Nussbaum, *ibid*. 7.

The tension of the carbon dioxide in the blood has been determined in different ways by **Pflüger** and his pupils, **Wolffberg**, **Strassburg**, and **Nussbaum**.[1]

According to the aërotonometric method the blood is allowed to flow directly from the artery or vein through a glass tube which contains a gas mixture of a known composition. If the tension of the carbon dioxide in the blood is greater than the gas mixture, then the blood gives up carbon dioxide, while in the reverse case it takes up carbon dioxide from the gas mixture. The analysis of the gas mixture after passing the blood through it will also decide if the tension of the carbon dioxide in the blood is greater or less than in the gas mixture; and by a sufficiently great number of determinations, especially when the quantity of carbon dioxide of the gas mixture corresponds as nearly as possible in the beginning to the probable tension of this gas in the blood, we may learn the tension of the carbon dioxide in the blood.

According to this method the carbon-dioxide tension of the arterial blood is on an average 2.8 per cent of an atmosphere, corresponding to a pressure of 21 mm. mercury (**Strassburg**). In the blood from the pulmonary alveoli **Nussbaum** found a carbon-dioxide tension of 3.81 per cent of an atmosphere, corresponding to a pressure of 28.95 mm. mercury. **Strassburg**, who experimented in non-tracheotomized dogs in which the ventilation of the lungs was less active and therefore the carbon dioxide was removed from the blood with less readiness, found in the venous blood of the heart a carbon-dioxide tension of 5.4 per cent of an atmosphere, corresponding to a partial pressure of 41.01 mm. mercury.

Another method is the catheterization of a lobe of the lungs (see page 708). In the air thus obtained from the lungs **Nussbaum** and **Wolffberg** found an average of 3.6 per cent CO_2. **Nussbaum**, as previously mentioned, has also determined the carbon-dioxide tension in the blood of the pulmonary alveoli in a case simultaneously with the catheterization of the lungs. He found nearly identical results, namely, a carbon-dioxide tension of 3.84 per cent and 3.81 per cent.

According to these investigations the giving up of carbon dioxide may also be explained by physical laws; but **Bohr**, in his experiments above mentioned (page 706), has arrived at other results in regard to the carbon-dioxide tension. In eleven experiments with inhalation of atmospheric air the carbon-dioxide tension in the arterial blood varied from 0 to 38 mm. Hg, and in five experiments with inhalation of air containing carbon dioxide, from 0.9 to 57.8 mm. Hg. A comparison of the carbon-dioxide tension in the blood with the bifurcated air gave in several cases a greater carbon-dioxide pressure in the air of the lungs than in the blood, and as maximum this difference amounted to 17.2 mm. in favor of the air of the lungs in the experiments with inhalation of atmospheric air. As the alveolar air is richer in carbon dioxide than the bifurcated air this experiment unquestionably proves, according to Bohr, that the carbon dioxide has migrated against the high pressure.

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[1] Wolffberg, Pflüger's Arch., 6; Strassburg, *ibid.*; Nussbaum, *ibid.*, 7.

In opposition to these investigations, Fredericq,[1] in his above-mentioned experiments, obtained the same figures for the carbon-dioxide tension in arterial peptone blood as Pflüger and his pupils found for normal blood. Weisgerber,[2] in Fredericq's laboratory, has made experiments with animals which respired air rich in carbon dioxide, and these experiments confirm Pflüger's theory of respiration. Recently Falloise has made determinations of the carbon-dioxide tension of venous blood by means of Fredericq's aërotonometer. The carbon-dioxide tension was found to equal 6 per cent of an atmosphere, hence somewhat higher than the results found by Pflüger's pupils. In opposition to these investigations Bohr has presented strong objections; he has demonstrated the principles for the construction of the tonometer and according to him the older experiments with the tonometer are not conclusive as he claims that a complete equilibrium of the gas tension was not sufficiently accomplished.

A certain importance has been ascribed to oxygen in regard to the elimination of carbon dioxide in the lungs, in that it has an expelling action on the carbon dioxide from its combinations in the blood. This statement, first made by Holmgren, has recently found an advocate in Werigo. Still Zuntz has presented very important objections to Werigo's experiments, and Bohr[3] has later also shown that we have no positive basis for the above assumption.

The conditions as to the elimination of carbon dioxide in the lungs is also not quite clear, and from the above we see that in regard to the gas exchange in the lungs we have two opposed views. According to the older view suggested by the Pflüger school the exchange of gas follows the simple physical laws and is on the whole a diffusion process. According to Bohr's view a diffusion does take place; but according to him the lung is a gland which has the power of secreting gases, and the gas exchange in the lungs is essentially a secretory process. According to Hammarsten we cannot dispute the fact that the investigations made thus far speak very much in favor of Bohr's view, and this latter also receives support in the detectable secretion of gases in certain animals.

That a true secretion of gases occurs in animals follows from the composition and behavior of the gases in the swimming-bladder of fishes. These gases consist of oxygen and nitrogen with only small quantities of carbon dioxide. In fishes which do not live at any great depth the quantity of oxygen is ordinarily as high as in the atmosphere, while fishes which live at great depths may, according to **Biot** and others, contain considerably more oxygen and even above 80 per cent. **Moreau** has also found that after emptying the swimming-bladder by means of a trocar new air collected after a time, and this air was richer in oxygen than the atmospheric air and contained even 85 per cent oxygen. **Bohr**, who has proved and confirmed these statements, also found that this collection is under the influence of the nervous system, because on the section of certain branches of the pneumogastric nerve it is discontinued. It is beyond dispute that there is here a secretion and not a diffusion of oxygen. Recently **Jaeger**[1] has given a further explanation as to the secretory activity of the swimming-bladder.

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[1] See footnote 3 page 706.

[2] Centralbl. f. Physiol. **10**, 482; Falloise, see Maly's Jahresber, **32**.

[3] Holmgren. Wien Sitzungsber., **48** Werigo, Pflüger's Arch., **51** and **52**; Zuntz, *ibid.*, **52**; Bohn, see Nagel's Handbuch der Physiologie.

From what has been said above (page 703) in regard to the internal respiration, one can conclude that it consists chiefly in that in the capillaries the oxygen passes from the blood into the tissues, while the carbon dioxide passes from the tissues into the blood.

The assertion of **Estor** and **Saint Pierre** that the quantity of oxygen in the blood of the arteries decreases with the remoteness from the heart has been shown to be incorrect by **Pflüger**,[2] and the oxygen tension in the blood on entering the capillaries must be higher. The oxygen tension of the plasma is of importance for the giving up of oxygen to the tissues as the blood corpuscles only contain a supply of oxygen, which, as the tissue removes oxygen from the plasma, replaces this again. This quantity of oxygen which is dissolved in the plasma and at the disposal of the tissues is dependent upon the oxygen tension in the blood and only indirectly dependent upon the total quantity of oxygen in the blood. As this tissue is nearly or entirely free from oxygen a considerable difference in regard to the oxygen pressure must exist between the blood and the tissues. The possibility that this difference in pressure is sufficient to supply the tissues with the necessary quantity of oxygen is hardly to be doubted.

The animal body it seems also has the command over means of regulating and varying the oxygen tension, and such a means is the carbon dioxide produced in the tissue which, according to **Bohr**, **Hasselbach**, and **Krogh**,[3] raises the oxygen tension. Another regulating moment is, according to **Bohr**, the *specific oxygen capacity* of the blood which means the relationship of the maximum oxygen combination to the quantity of iron of the blood or the hæmoglobin solution.

As the hæmoglobin obtained from different blood portions does not, according to **Bohr**, always take up the same quantity of oxygen for each gram, so the hæmoglobin within the blood-corpuscle may show a similar behavior. He calls the quantity of oxygen (measured at 0° C. and 760 mm. Hg which is taken up by 1 gram of hæmoglobin of the blood at 15° C. and an oxygen pressure of 150 mm. the *specific oxygen capacity*.[4] This quantity, he claims, may be different not only in different individuals, but also in the different vascular systems of the same animal, and it may also be changed experimentally by bleeding, breathing air deficient in oxygen, or poisoning. It is now evident that one and the same quantity of oxygen in the blood, other things being equal, must have a different tension according as the specific oxygen capacity is greater or smaller. The tension of the oxygen, BOHR says, may be changed without changing the quantity of oxygen, and the animal body must, according to him, have means of varying the tension of the oxygen in the tissues in a short time without changing the quantity of oxygen contained in the blood. The great importance of such a property of the tissues for respiration is evident; but it is perhaps too early to give a positive opinion on BOHR's statements and experiments.

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[1] Biot, see Hermann's Handbuch d. Physiol., 4, Thl. 2, 151; Moreau, Compt. rend., 57; Bohr, Journ. of Physiol., 15. See also Hüfner, Arch. f. (Anat. u.) Physiol., 1892; Jaeger, Pflüger's Arch., 94.

[2] Estor and Saint Pierre with Pflüger in Pflüger's Arch. 1.

[3] L. c.

[4] Centralbl. f. Physiol. 4 and Nagel's Handbuch.

In regard to the carbon-dioxide tension in the tissue it must be assumed *à priori* that it is higher than in the blood. This is found to be true. STRASSBURG[1] found in the urine of dogs and in the bile a carbon-dioxide tension of 9 per cent and 7 per cent of an atmosphere, respectively. The same experimenter has, further, injected atmospheric air into a ligatured portion of the intestine of a living dog and analyzed the air taken out after some time. He found a carbon-dioxide tension of 7.7 per cent of an atmosphere. The carbon-dioxide tension in the tissues is considerably greater than in the venous blood, and there is no opposition to the view that the carbon dioxide simply diffuses from the tissues into the blood according to the laws of diffusion.

Several methods have been suggested for the study of the quantitative relationship of the respiratory exchange of gas. The reader must be referred to other text-books for more details as to these methods, and we will here only mention the chief features of the most important methods.

REGNAULT and REISET's *Method.* According to this method the animal or person experimented upon is allowed to respire in an enclosed space. The carbon dioxide is removed from the air, as it forms, by strong caustic alkali, from which the quantity may be determined, while the oxygen is replaced continually by exactly measured quantities. This method, which also makes possible a direct determination of the oxygen used as well as the carbon dioxide produced, has since been modified by other investigators, such as PFLÜGER and his pupils, SEEGEN and NOWAK, and HOPPE-SEYLER, ROSENTHAL, and ZUNTZ.[2]

PETTENKOFER's *Method.* According to this method the individual to be experimented upon breathes in a room through which a current of atmospheric air is passed. The quantity of air passed through is carefully measured. As it is impossible to analyze all the air made to pass through the chamber, a small fraction of this air is diverted into a branch line during the entire experiment, carefully measured, and the quantity of carbon dioxide and water determined. From the composition of this air the quantity of water and carbon doxide contained in the large quantity of air made to pass through the chamber can be calculated. The consumption of oxygen cannot be directly determined in this method, but may be calculated indirectly by difference, which is a defect in this method. The large respiration apparatus of Sondén and Tigerstedt as well as of Atwater and Rosa[1] are based upon this principle.

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[1] Pflüger's Arch. **6**.

[2] See Zuntz in Hermann's Handbuch, **4**, Thl. 2, and Hoppe-Seyler, Zeitschr. f. physiol. Chem., **19**; Rosenthal, Arch. f. (Anat. u.) Physiol., 1902; Zuntz, Verhandl. d. Berl. physiol. Gesellsch., 1901.

Speck's *Method.*[2] For briefer experiments on man Speck has used the following: He breathes into two spirometer-receivers, on which the gas-volume can be read off very accurately, through a mouthpiece with two valves, closing the nose with a clamp. The air from one of the spirometers is inhaled through one valve and the expired air passes through the other into the other spirometer. By means of a rubber tube connected with the expiration-tube an accurately measured part of the expired air may be passed into an absorption-tube and analyzed.

Zuntz and Geppert's *Method.*[3] This method, which has been improved by Zuntz and his pupils from time to time, consists in the following: The individual being experimented upon inspires pure atmospheric air through a very wide feed-pipe leading from the open air, the inspired and the expired air being separated by two valves (human subjects breathe with closed nose by means of a soft-rubber mouthpiece, animals through an air-tight tracheal canula). The volume of the expired air is measured by a gas-meter and an aliquot part of this air collected and the quantity of carbon dioxide and oxygen determined. As the composition of the atmospheric air can be considered as constant within a certain limit, the production of carbon dioxide as well as the consumption of oxygen may be readily calculated (see the works of Zuntz and his pupils).

Hanriot and Richet's *Method*[4] is characterized by its simplicity. These investigators allow the total air to pass through three gasometers, one after the other. The first measures the inspired air, whose composition is known. The second gasometer measures the expired air, and the third the quantity of the expired air after the carbon dioxide has been removed by a suitable apparatus. The quantity of carbon dioxide produced and the oxygen consumed can be readily calculated from these data.

APPENDIX.

The Lungs and their Expectorations.

Besides *proteid bodies* and the *albuminoids* of the connective-substance group, *lecithin, taurine* (especially in ox-lungs), *uric acid,* and *inosite* have been found in the lungs. Poulet[5] claims to have found a special acid, which he has called *pulmotartaric acid,* in the lung-tissue. Glycogen occurs abundantly in the embryonic lung, but is absent in the adult organ. The proteolytic enzymes also belong to the physiological constituents of the lungs. They are active in the autolysis of the lungs (Jacoby) as well as in the solution of pneumonic infiltrations (Fr. Muller[6]).

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[1] Pettenkofer's method; see Zuntz, l. c.; Sondén and Tigerstedt, Skand. Arch. f. Physiol., 6; Atwater and Rosa, Bull. of Dept. of Agriculture, 63. Washington.

[2] Speck, Physiologie des menschlichen Atmens. Leipzig, 1892.

[3] Pflüger's Arch., 42. See also Magnus-Levyin Pflüger's Arch., 55, 10, in which the work of Zuntz and his pupils is cited.

[4] Compt. rend., 104.

[5] Cited from Maly's Jahresber., 18, 248.

[6] Jacoby, Zeitschr. f. physiol. Chem., 33; Müller, Verhandl. d. Kongress. f. inn. Medizin, 1902.

The black or dark-brown pigment in the lungs of human beings and domestic animals consists chiefly of carbon, which originates from the soot in the air. The pigment may in part also consist of melanin. Besides carbon, other bodies, such as iron oxide, silicic acid, and clay, may be deposited in the lungs, being inhaled as dust.

Among the bodies found in the lungs under pathological conditions must be specially mentioned proteoses (and peptones?) in pneumonia and suppuration, glycogen, a slightly dextrorotatory carbohydrate differing from glycogen found by POUCHET in consumptives, and finally also cellulose, which, according to FREUND,[1] occurs in the lungs, blood, and pus of persons with tuberculosis.

C. W. SCHMIDT found in 1000 grams of mineral bodies from the normal human lung the following: NaCl 130, K_2O 13, Na_2O 195, CaO 19, MgO 19, Fe_2O_3 32, P_2O_5 485, SO_3 8, and sand 134 grams. According to OIDTMANN[2] the lungs of a 14-day old child contained 796.05 p. m. water, 198.19 p. m. organic bodies, and 5.76 p. m. inorganic bodies.

The sputum is a mixture of the mucous secretion of the respiratory passages, of saliva and buccal mucus. Because of this its composition is very variable, especially under pathological conditions when various products mix with it. The chemical constituents are, besides the mineral substances, chiefly mucin with a little proteid and nuclein substance. Under pathological conditions proteoses and peptone (?), which are probably produced by bacterial action or by autolysis (WANNER, SIMON[3]), volatile fatty acids, glycogen, CHARCOT's crystals, and also crystals of cholesterin, hæmatoidin, tyrosine, fat and fatty acids, triple phosphates, etc., have been found.

The form constituents are, under physiological circumstances, epithelium-cells of various kinds, leucocytes, sometimes also red blood-corpuscles and various kinds of fungi. In pathological conditions elastic fibres, spiral formations consisting of a mucin-like substance, fibrin coagulum, pus, pathogenic microbes of various kinds, and the above-mentioned crystals occur.

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[1] Pouchet, Compt. rend., **96**; Freund, cited from Maly's Jahresber, **16**, 471.

[2] Schmidt, cited from v. Gorup-Besanez, Lehrbuch, 4. Aufl., 727; Oidtmann, *ibid.*, 732.

[3] Wanner, Deutsch. Arch. f. klin. Med., **75**; Simon, Arch. f. exp. Path. u. Pharm., **49**.

CHAPTER XVIII.

METABOLISM WITH VARIOUS FOODS, AND THEIR NECESSITY TO MAN.

THE conversion of chemical energy into heat and mechanical work which characterizes animal life, leads, as previously stated in Chapter I, to the formation of relatively simple compounds — carbon dioxide, urea, etc. — which leave the organism, and which, moreover, being very poor in energy, are for this reason of little or no value for the body. It is therefore absolutely necessary for the continuance of life and the normal course of the functions of the body that the organism and its different tissues should be supplied with new material to replace that which has been exhausted. This is accomplished by means of food. Those bodies are designated as *food* which have no injurious action upon the organism and which serve as a source of energy and can replace those constituents of the body that have been consumed in metabolism or that can prevent or diminish the consumption of such constituents.

Among the numerous dissimilar substances which man and animals take with the food all cannot be equally necessary or have the same value. Some perhaps are unnecessary, while others may be indispensable. We have learned by direct observation and a wide experience that besides the oxygen, which is necessary for oxidation, the essential foods for animals in general, and for man especially, are *water*, *mineral bodies*, *proteins*, *carbohydrates*, and *fats*.

It is also apparent that the various groups of foodstuffs necessary for the tissues and organs must be of varying importance; thus, for instance, water and the mineral bodies have another value than the organic foods, and these again must differ in importance among themselves. The knowledge of the action of various nutritive bodies on the exchange of material from a qualitative as well as a quantitative point of view must be of fundamental importance in determining the value of different nutritive substances relative to the demands of the body for food under various conditions, and also in deciding many other questions — for instance, the proper nutrition for an individual in health and in disease.

Such knowledge can only be attained by a series of systematic and thorough observations, in which the quantity of nutritive material, relative to the weight of the body, taken and absorbed in a given time is compared with the quantity of final metabolic products which leave the organism at the same time. Researches of this kind have been made by several investigators, but above all should be mentioned those made by BISCHOFF and VOIT, by PETTENKOFER and VOIT, and by VOIT and his pupils, by RUBNER and by ATWATER.

It is absolutely necessary in researches on the exchange of material to be able to collect, analyze, and quantitatively estimate the excreta of the organism, so that they may be compared with the quantity and composition of the nutritive bodies ingested. In the first place, one must know what the habitual excreta of the body are and in what way these bodies leave the organism. One must also have trustworthy methods for the quantitative estimation of the same.

The organism may, under physiological conditions, be exposed to accidental or periodic losses of valuable material — such losses as only occur in certain individuals, or in the same individual only at a certain period; for instance, the secretion of milk, the production of eggs, the ejection of semen or menstrual blood. It is therefore apparent that these losses can be the subject of investigation and estimation only in special cases.

The regular and constant excreta of the organism are of the very greatest importance in the study of metabolism. To these belong, in the first place, the true final metabolic products — *carbon dioxide, urea* (uric acid, hippuric acid, creatinine, and other urinary constituents), and a part of the *water*. The remainder of the water, the mineral bodies, and those secretions or tissue constituents — *mucus, digestive fluids, sebum, perspiration*, and *epidermal formations* — which are either poured into the intestinal tract, or secreted from the surface of the body, or broken off and thereby lost to the body, also belong to the constant excreta.

The remains of food, sometimes indigestible, sometimes digestible but not acted upon, which are contained in the fæces, and which vary considerably in quantity and composition with the nature of the food, also belong to the excreta of the organism. Even though these remains, which are never absorbed and therefore are never constituents of the animal fluids or tissues, cannot be considered as excreta of the body in a strict sense, still their quantitative estimation is absolutely necessary in certain experiments on the exchange of material.

The determination of the constant loss is in some cases accompanied with the greatest difficulties. The loss from the detached epidermis, from the secretion of the sebaceous glands, etc., cannot be determined with exactness without difficulty, and therefore — as they do not occasion any appreciable loss because of their small quantity — they need not be considered in quantitative experiments on metabolism. This also applies to the constituents of the mucus, bile, pancreatic and intestinal juices, etc., occurring in the contents of the intestine, and which, leaving the body with the fæces, cannot be separated from the other contents of the intestine and therefore cannot be quantitatively determined separately. The uncertainty which because of the intimated difficulties, attaches itself to the results of the experiment, is very small as compared to the variation which is caused by different individualities, different modes of living, different foods, etc. No general but only approximate values can therefore be given for the constant excreta of the human body.

The following figures represent the quantity of excreta for twenty-four hours from a grown man, weighing 60–70 kilos, on a mixed diet. The numbers are compiled from the results of different investigators.

	Grams.
Water	2500–3500
Salts (with the urine)	20–30
Carbon dioxide	750–900
Urea	20–40
Other nitrogenous urinary constituents	2–5
Solids in the excrements	20–50

These total excreta are approximately divided among the various excretions in the following way; but still it must not be forgotten that this division may vary to a great extent under various external circumstances: by *respiration* about 32 per cent, by the *evaporation from the skin* 17 per cent, with the *urine* 46–47 per cent, and with the *excrements* 5–9 per cent. The elimination by the skin and lungs, which is sometimes differentiated by the name "*perspiratio insensibilis*" from the visible elimination by the kidneys and intestine, is on an average about 50 per cent of the total elimination. This proportion, quoted only relatively, is subject to considerable variation, because of the great difference in the loss of water through the skin and kidneys under different circumstances.

The nitrogenous constituents of the excretions consist chiefly of urea, or uric acid in certain animals, and the other nitrogenous urinary constituents. A disproportionately large part of the nitrogen leaves the body with the urine, and, as the nitrogenous constituents of this excretion are final products of the metabolism of proteins in the organism, the quantity of proteins catabolized in the body may be easily calculated by multiplying the quantity of nitrogen in the urine by the coefficient 6.25 ($\frac{100}{16} = 6.25$), if it is admitted that the proteins contain in round numbers 16 per cent of nitrogen.

Still another question is whether the nitrogen leaves the body only with the urine or by other channels. The latter is habitually the case. The discharges from the intestine always contain some nitrogen, which as stated in Chapter IX consists in part of non-absorbed remnants of the food, but in chief part and sometimes entirely of constituents of the epithelium and the secretions. Under these circumstances it is apparent that one cannot give any exact figures which are valid for all cases for that part of the nitrogen of the excrements which originates from the digestive tract and from the digestive fluids. It may not only vary in different individuals, but also in the same individual after more or less active secretion and absorption. In the attempts made to determine this part of the nitrogen of the excrements it has been found that in man, on non-nitrogenous or nearly nitrogen-free food, it amounts in round numbers to somewhat less than 1 gram per twenty-four hours (RIEDER, RUBNER). Even with such food the absolute quantity of nitrogen eliminated by the fæces increases with the quantity of food because of the accelerated digestion (TSUBOI [1],) and is greater than in starvation. MULLER [2] found in his observations on the faster CETTI that only 0.2 gram nitrogen was derived from the intestinal canal.

The quantity of nitrogen which leaves the body under normal circumstances by means of the hair and nails, with the scaling off of the skin, and with the perspiration cannot be accurately determined. It is nevertheless so small that it may be ignored. Only in profuse sweating need the elimination by this channel be taken into consideration.

The view was formerly held that in man and carnivora an elimination of gaseous nitrogen took place through the skin and lungs, and because of this, on comparing the nitrogen of the food with that of the urine and fæces, a *nitrogen deficit* occurred in the visible elimination.

This question has been the subject of much discussion and of numerous investigations.[3] These investigations have shown that the above assumption is unfounded, and moreover several investigators, especially PETTENKOFER and VOIT, and GRUBER,[4] have shown by experiments on man and animals that with the proper quantity and quality of food the body can be brought into *nitrogenous equilibrium*, in which the quantity of nitrogen voided with the urine and fæces is equal or nearly equal to the quantity contained in the food. Undoubtedly we must admit with VOIT that a deficit of nitrogen does not exist, or it is so insignificant that in experiments upon metabolism it need not be considered. Ordinarily, in investigations on the catabolism of proteins in the body, it is only necessary to consider the nitrogen of the urine and fæces, but it must be remarked that the nitrogen of the urine is a measure of the extent of the catabolism of the proteins in the body, while the nitrogen of the fæces (after deducting about 1 gram on a mixed diet) is a measure of the non-absorbed part of the nitrogen of the food. The nitrogen of the food, as well as of the excreta, is generally determined by KJELDAHL's method.

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[1] Rieder, Zeitschr. f. Biologie, **20**; Rubner, *ibid.*, **15**; Tsuboi, *ibid.*, **35**.

[2] Berlin. klin. Wochenschr., 1887.

[3] See Regnault and Reiset, Annal. d. chim. et phys. (3), **26**, and Annal. d. Chem. u. Pharm., **73**; Seegen and Nowak, Wien. Sitzungsber., **71**, and Pflüger's Arch., **25**; Pettenkofer and Voit, Zeitschr. f. Biologie, **16**; Leo, Pflüger's Arch., **26**.

[4] Pettenkofer and Voit, in Hermann's Handbuch, **6**, Thl. 1; Grüber, Zeitschr. f. Biologie, **13** and **19**.

In the oxidation of the proteins in the organism their sulphur is oxidized into sulphuric acid, and on this depends the fact that the elimination of sulphuric acid by the urine, which in man is only to a small extent derived from the sulphates of the food, makes nearly equal variations with the elimination of nitrogen by the urine. If the amount of nitrogen and sulphur in the proteins is considered as 16 per cent and 1 per cent respectively, then the proportion between the nitrogen of the proteins and the sulphuric acid, H_2SO_4, produced by their combustion is in the ratio 5.2:1, or about the same as in the urine (see page 622). The determination of the quantity of sulphuric acid eliminated in the urine gives us an important means of controlling the extent of the transformation of proteins, and such a control is especially important in cases in which it is expected to study the action of certain nitrogenous non-albuminous bodies on the metabolism of proteins. A determination of the nitrogen alone is not sufficient in such cases. A perfectly positive measure of the protein catabolism cannot be made from the sulphuric acid of the urine, as the various protein substances have a rather variable sulphur content, and on the other hand also a variable quantity of the sulphur in the urine exists as so-called neutral sulphur.

In metabolism experiments the total sulphur of the urine as well as the fæces must be determined. The sulphur of the catabolized proteins is quicker eliminated, according to v. WENDT, than the nitrogen, and this behavior of sulphur gives a more positive picture of the temporal catabolism of protein than the nitrogen. This is all the more important as according to FALTA[1] not only does the nitrogen corresponding to a certain amount of protein require several days for elimination but also the chief quantity of this nitrogen in man after taking different kinds of proteins is eliminated with varying rapidity.

Besides lecithins and other phosphatides the body takes with its food pseudonucleins as well as true nucleins and these are absorbed more or less completely from the intestinal tract and then assimilated (GUMLICH, SANDMEYER, MARCUSE, ROHMANN, and STEINITZ, LOEWI,[2] and others). On the other hand, the phosphorized protein substances, lecithins and phosphatides, are also decomposed within the body, and their phosphorus is chiefly eliminated as phosphoric acid and also in part as organic phosphorus (see page 619). For these reasons the phosphorus is of great importance in certain investigations on metabolism.

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[1] v. Wendt, Skand. Arch. f. Physiol., **17**; Falta, Deutsch. Arch. f. klin. Med. **86**.

[2] In regard to the investigations on the metabolism of phosphorus and the methods used therein, see Steinitz, Pflüger's Arch., **72**; Zadik, *ibid.*, **77**; Leipziger, *ibid.*, **78**; Oertel Zeitschr. f. physiol. Chem., **26**; Mandel and Oertel, Bull. Med. Sciences, N. Y. Univ., **1**, and Ehrlich, Inaug.-Diss., Breslau, 1900; Loewi, Arch. f. exp. Path. u. Pharm., **45**. On the absorption of casein, see Poda, Prausnitz, Micko, and P. Müller, Zeitschr. f. Biologie, **39**. The literature on the phosphorus metabolism can be found in Albu and Neuberg, Physiol. u. Pathol. des Mineralstoffwechsels, Berlin, 1906.

If it is found, on comparing the nitrogen of the food with that of the urine and fæces, that there is an excess of the first, this means that the body has increased its stock of nitrogenous substances — proteins. If, on the contrary, the urine and fæces contain more nitrogen than the food taken at the same time, this denotes that the body is giving up part of its nitrogen — that is, a part of its own proteins has been decomposed. We can, from the quantity of nitrogen, as above stated, calculate the corresponding quantity of proteins by multiplying by 6.25.[1] Usually, according to VOIT's proposition, the nitrogen of the urine is not calculated as decomposed proteins, but as decomposed muscle-substance or flesh. Lean meat contains on an average about 3.4 per cent nitrogen; hence each gram of nitrogen of the urine corresponds in round numbers to about 30 grams of flesh. The assumption that lean meat contains 3.4 per cent nitrogen is arbitrary, and the relationship of N: C in the proteins of dried meat, which is of great importance in certain experiments on metabolism, is given differently by various experimenters, namely, 1:3.22 — 1:3.68. ARGUTINSKY found in beef, after complete removal of fat and subtraction of glycogen, that the relationship was 1:3.24 (see Chapter XI).

The carbon leaves the body chiefly as carbon dioxide, which is eliminated by the lungs and skin. The remainder of the carbon is excreted in the urine and fæces in the form of organic compounds, in which the quantity of carbon can be determined by elementary analysis. It used to be considered sufficient to calculate the quantity of carbon in the urine from the quantity of nitrogen according to the relationship N: C = 1: 0.67. This does not seem to be trustworthy, as this relationship varies and depends, according to TANGL and PFLÜGER, LANGSTEIN, and STEINITZ,[2] upon the kind of food. TANGL has shown that the richer the food is in carbohydrates the more carbon and heat of combustion per gram of nitrogen does the urine contain. He found the following for 1 gram of nitrogen in the urine: With diet rich in fat 0.747 gram C and 9.22 Calories; for carbohydrate-rich diet he found 0.963 gram C and 11.67 Calories.

The quantity of gaseous carbon dioxide eliminated may be determined by means of PETTENKOFER's respiration apparatus or by other methods. By multiplying the quantity of carbon dioxide found by 0.273 one obtains the quantity of carbon eliminated as CO_2. If the total quantity of carbon eliminated in various ways is compared with the carbon contained in the food some idea can be obtained as to the transformation of the carbon compounds. If the quantity of carbon in the food is greater than in the excreta, then the excess is deposited; while if the reverse be the case it shows a corresponding loss of body substance.

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[1] In calculating the protein catabolism from the nitrogen of the urine it must not be forgotten that the food often contains nitrogenous extractions whose nitrogen cannot be calculated as protein and for which a special correction must be made, if necessary.

[2] Tangl, Arch. f. (Anat. u.) Physiol., 1899, Supplbd.; Pflüger in Pflüger's Arch., **79**; Langstein and Steinitz, Centralbl. f. Physiol., **19**.

The nature of the substances here deposited or lost, whether they consist of proteins, fats, or carbohydrates, is learned from the total quantity of the nitrogen of the excretions. The corresponding quantity of proteins may be calculated from the quantity of nitrogen, and, as the average quantity of carbon in the proteins is known, the quantity of carbon which corresponds to the decomposed proteins may be easily ascertained. If the quantity of carbon thus found is smaller than the quantity of the total carbon in the excreta, it is then obvious that some other nitrogen-free substance has been consumed besides the proteins. If the quantity of carbon in the proteins is considered in round numbers as 53 per cent,[1] then the relation between carbon (53) and nitrogen (16) is as 3.3 : 1. If the total quantity of nitrogen eliminated is multiplied by 3.3, the excess of carbon in the eliminations over the product found represents the carbon of the decomposed non-nitrogenous compounds. For instance, in the case of a person experimented upon, 10 grams of nitrogen and 200 grams of carbon were eliminated in the course of 24 hours; then these 62.5 grams of protein correspond to 33 grams of carbon, and the difference, $200-(3.3\times 10)=167$, represents the quantity of carbon in the decomposed non-nitrogenous compounds. If we start from the simplest case, starvation, where the body lives at the expense of its own substance, then, since the quantity of carbohydrates as compared with the fats of the body is extremely small, in such cases in order to avoid mistakes the assumption must be made that the person experimented upon has used only fat and proteins. As animal fat contains on an average 76.5 per cent carbon, the quantity of transformed fat may be calculated by multiplying the carbon by $\frac{100}{76.5}=1.3$. In the case of the above example, the person experimented upon would have used 62.5 grams of proteins and $167\times 1.3=217$ grams of fat of his own body in the course of the twenty-four hours.

Starting from the nitrogen balance, it can be calculated in the same way whether an excess of carbon in the food as compared with the quantity of carbon in the excreta is retained by the body as proteins or fat or as both. On the other hand, with an excess of carbon in the excreta one can determine how much of the loss of the substance of the body is due to a consumption of the proteins or of fat or of both.

The quantity of water and mineral bodies voided with the urine and fæces can easily be determined. The quantity of water eliminated by the skin and lungs may be directly estimated by means of PETTENKOFER's apparatus. The quantity of oxygen taken up is calculated as the difference between the weight of the individual before the experiment plus all the directly determined substances ingested, and the final weight of the individual plus all his excreta.

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[1] This figure is perhaps a little too high.

The oxygen may also be determined directly, according to REGNAULT-REISET's method, or in other ways, and such a determination with the simultaneous estimation of the carbon dioxide eliminated is of great importance in the study of metabolism.[1]

On comparing the inspired and the expired air we learn, on measuring them when dry and at the same temperature and pressure, that the volume of the expired air is less than that of the inspired air. This depends upon the fact that not all of the oxygen appears again in the expired air as carbon dioxide, because it is not only used in the oxidation of carbon, but also in part in the formation of water, sulphuric acid, and other bodies. The volume of expired carbon dioxide is regularly less than the volume of the inspired oxygen, and the relation $\frac{CO_2}{O}$, which is called the *respiratory quotient*, is generally less than 1.

The magnitude of the respiratory quotient is dependent upon the kind of substances destroyed in the body. In the combustion of pure carbon one volume of oxygen yields one volume of carbon dioxide, and the quotient is therefore equal to 1. The same is true in the burning of carbohydrates, and in the exclusive decomposition of carbohydrates in the animal body the respiratory quotient must be approximately 1. In the exclusive metabolism of proteins it is close to 0.80, and with the decomposition of fat it is 0.7. In starvation, as the animal draws on its own flesh and fat, the respiratory quotient must be a close approach to the latter figure. The respiratory quotient therefore gives important data on the quality of the material decomposed in the body, naturally with the supposition that the elimination of carbon dioxide, independent of the formation of carbon dioxide, is not influenced by special conditions, such as the alteration of the respiratory mechanism.

It is also possible in systematized experimentation to carry on the metabolism experiments so that the decomposable material of the body, as shown by the respiratory quotient, remains qualitatively the same, at least for a short time. In such experiments it has been shown, especially by Zuntz and his pupils,[1] that the extent of oxygen consumption may be taken as a measure for the action of different influences on the extent of metabolism. This possibility is based on the fact proved by Pflüger and his pupils, and by Voit,[2] that the consumption of oxygen within wide limits is independent of the supply of oxygen, and is exclusively dependent upon the oxygen demand of the tissues. For certain reasons the consumption of oxygen gives indeed a better conclusion than the elimination of carbon dioxide as to the extent of exchange of material and energy; but as the same quantity of oxygen (100 grams) consumes different quantities of fat, carbohydrates, and proteins in the body — namely, 35, 84.4, and 74.4 grams respectively — the respiratory quotient must also be determined, in order to ascertain the nature of the substance burnt in the body, simultaneously with the determination of the carbon dioxide.

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[1] In regard to the methods for estimating the carbon-dioxide excretion and the oxygen consumption, see Zuntz, Hermann's Handbuch d. Physiol., **4**, Tl. 2; Hoppe-Seyler, Zeitschr. f. physiol. Chem., **19**; Sondén and Tigerstedt, Skand. Arch. f. Physiol., **6**; Speck, Physiol. des menschl. Atmens. Leipzig, 1892; Zuntz and Geppert, Pflüger's Arch., **42**; Magnus-Levy, *ibid.*, **55**, 10, where the works of Zuntz and his pupils are cited; Hanriot et Richet, Compt. rend., **104**, and Atwater, Bull. of Dept. of Agric., Washington, Nos. **44**, **63**, **69**, and **109**.

As the different foods require different amounts of oxygen in the combustion of each gram of substance and yield different amounts of CO_2, each gram of oxygen taken up and each gram of carbon in the expired air as carbon dioxide must correspond to different heat values. This follows from the following table:

	Calories per grm. C in the CO_2 of the Expired Air.	Relative Value.	Calories per grm. Consumed Oxygen.	Relative Value.
In the combustion of cane-sugar	9.5	100	3.56	118.6
" " " " meat	10.2	107	3.00	100.0
" " " " fat	12.3	129	3.27	109.0

Pflüger has found the following figures for the calorific value of 1 gram oxygen:

For muscle tissue free from fat	3.30 Cal.
Fat	3.29 "
Starch	3.53 "

The figures for the oxygen differ, as seen above, less than those for the carbon, and this is the reason why, as above stated, the oxygen consumption gives a much more correct conclusion as to the exchange of force than the elimination of carbon dioxide.[3]

Kaufmann[4] encloses the individual to be experimented upon in a capacious sheet-iron room, which serves both as a respiration-chamber and a calorimeter, and which permits of the estimation of the nitrogen of the urine and the carbon dioxide expired, as well as the inspired oxygen and the quantity of heat produced. If we start from the theoretically calculated formulæ for the various possible transformations of the proteins, fats, and carbohydrates in the body, it is clear that other values must be obtained for the heat, carbon dioxide, oxygen, and nitrogen of the urine, when one, for example, admits of a complete combustion of proteins to urea, carbon dioxide, and water, or of a partial splitting off of fat. Another relationship between heat, carbon dioxide, and oxygen is also to be expected when the fat is completely burnt or when it is decomposed into sugar, carbon dioxide, and water. In this way, by a comparison of the values found in special cases with the figures calculated for the various transformations, KAUFMANN attempts to explain the various decomposition processes in the body under different nutritive conditions.

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[1] See footnote, page 722.

[2] Pflüger, Pflüger's Arch., **6**, **10**, and **14**; Finkler, *ibid.*, **10**; Finkler and Oertmann, *ibid.*, **14**; Voit, Zeitschr, f. Biologie, **11** and **14**.

[3] See Ad. Magnus-Levy, Pflüger's Arch., **55**, **7**, and Pflüger, *ibid.*, **77**, **78**, and **79**.

[4] Arch. d. Physiologie (5), **8**.

I. The Energy and the Relative Nutritive Value of Various Organic Foodstuffs.

With the organic foods the organism receives a supply of chemical energy which is converted into heat and mechanical work in the body. This energy of the various foods may be represented by the amount of heat which is set free in their combustion. This quantity of heat is expressed as calories, and a small calorie is the quantity of heat necessary to warm 1 gram of water from 0° to 1° C. A large calorie is the quantity of heat necessary to warm 1 kilo of water 1° C. Here and in the following pages large calories are to be understood. There are numerous investigations by different experimenters, such as FRANKLAND, DANILEWSKI, RUBNER, BERTHELOT, STOHMANN, and others, on the calorific value of different foodstuffs. The following results, which represent the calorific value of a few nutritive bodies on complete combustion outside of the body to the highest oxidation products, are taken from STOHMANN's[1] work.

	Calories.
Casein	5.86
Ovalbumin	5.74
Conglutin	5.48
Protein (average)	5.71
Animal tissue-fat	9.50
Butter-fat	9.23
Cane-sugar	3.96
Milk-sugar	3.95
Dextrose	3.74
Starch	4.19

Fats and carbohydrates are completely burnt in the body, and one can therefore consider their combustion equivalent as a measure of the living force developed by them within the organism. We generally designate 9.3 and 4.1 calories for each gram of substance as the average for the physiological calorific value of fats and carbohydrates respectively.

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[1] See Rubner, Zeitschr. f. Biologie, **21**, which also cites the works of Frankland and Danilewski; see also Berthelot, Compt. rend., **102, 104,** and **110;** Stohmann. Zeitschr. f. Biologie, **31.**

The proteins act differently from the fats and carbohydrates. They are only incompletely burnt, and they yield certain decomposition products, which, leaving the body with the excreta, still represent a certain quantity of energy which is lost to the body. The heat of combustion of the proteins is smaller within the organism than outside of it, and they must therefore be specially determined. For this purpose **Rubner**[1] fed a dog on washed meat, and he subtracted from the heat of combustion of the food the heat of combustion of the urine and fæces, which corresponded to the food taken plus the quantity of heat necessary for the swelling up of the proteins and the solution of the urea. **Rubner** has also tried to determine the heat of combustion of the proteins (muscle-proteins) decomposed in the body of rabbits in starvation. According to these investigations, the physiological heat of combustion in calories for each gram of substance is as follows:

1 grm. of the dry substance.	Calories.
Protein from meat	4.4
Muscle	4.0
Protein in starvation	3.8
Fat (average for various fats)	9.3
Carbohydrates (calculated average)	4.1

The physiological combustion value of the various foods belonging to the same group is not quite the same. It is, for instance, 3.97 calories for a vegetable protein, conglutin, and 4.42 calories for an animal protein body, syntonin. According to **Rubner** the normal heat value per 1 gram of animal protein may be considered as 4.23 calories, and of vegetable protein as 3.96 calories. When a person on a mixed diet takes about 60 per cent of the proteins from animal foods and about 40 per cent from vegetable foods, the value of 1 gram of the protein of the food is equivalent to about 4.1 calories. The physiological value of each of the three chief groups of organic foods, by their decomposition in the body, is in round numbers as follows:

	Calories.
1 gram protein	4.1
1 " fat	9.3
1 " carbohydrate	4.1

As will be shown, the fats and carbohydrates may decrease the metabolism of proteins in the body, while, on the other hand, the quantity of proteins in the body or in the food acts on the metabolism of fat in the body. In physiological combustion the various foods may replace one another to a certain extent, and it is therefore important to know the ratio of replacement. The investigations made by **Rubner** have taught that this, if it relates to the force and heat production in the animal body, is a proportion that corresponds with the figures of the heat value of the same. This is apparent from the following table. In this is found the weight of the various foods equal to 100 grams of fat, a part determined from experiments on animals and a part calculated from figures of the heat values.

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[1] Zeitschr. f. Biologie, **21**.

100 grams fat are equal to or isodynamic with

	From Experiments on Animals.	From the Heat Value.	Difference, per cent.
Syntonin	225	213	+5.6
Muscle-flesh (dried)	243	235	+4.3
Starch	232	229	+1.3
Cane-sugar	234	235	−0
Dextrose	256	255	−0

From the given *isodynamic value* of the various foods it follows that these substances replace one another in the body almost in exact ratio to the energy contained in them. Thus in round numbers 227 grams of protein and carbohydrate are equal to or isodynamic with 100 grams of fat in regard to source of energy, because each yields 930 calories on combustion in the body.

By means of recent very important calorimetric investigations **Rubner**[1] has shown that the heat produced in an animal in several series of experiments extending over forty-five days corresponded to within 0.47 per cent of the physiological heat of combustion calculated from the decomposed body and foods. **Atwater** and his collaborators [2] have made some very thorough investigations on this subject on men. In their experiments they made use of a large respiration calorimeter, which not only determined exactly the excreta but also made a calorimetric determination of the heat given out by the person experimented upon, i.e., the work performed. From the results of these experiments they found nearly an absolutely complete agreement between the calories found directly and those calculated.

This isodynamic law is of fundamental value in the study of metabolism and nutrition. By this law it is possible to consider the processes of metabolism as more uniform transformations of energy. The quantity of energy in the transformed foods or the constituents of the body may be used as a measure for the total consumption of energy, and the knowledge of the quantity of energy in the foods must also be the basis for the calculation of dietaries for human beings under various conditions.

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[1] Zeitschr. f. Biologie, **30**.

[2] Bull. of Dept. of Agric., Washington, **44**, **63**, **69**, and **109** and Ergebnisse des Physiologie **3**.

The heat value of a foodstuff can be directly determined in a calorimeter but may also be calculated from its composition. If one subtracts from the gross heat value of the food obtained in one way or another, the combustion heat of the fæces and urine with the same diet, there is obtained the net calorific value of the diet. This value, calculated in percentage of the total energy content of the food, is called the *physiological availability* by RUBNER.[1] In order to elucidate this we will give a few of RUBNER's values. The loss in calories, as well as the physiological availability, are calculated in percentages of the total energy content of the food.

Food.	Loss in per cent. In urine.	Loss in per cent. In the fæces.	Total loss in per cent.	Availability in per cent.
Cow's milk	5.13	5.07	10.20	89.8
Mixed diet (rich in fat)	3.87	5.73	9.60	90.4
" " (poor in fat)	4.70	6.00	10.70	89.3
Potatoes	2.00	5.60	7.60	92.4
Graham bread	2.40	15.50	17.90	82.1
Rye bread	2.20	24.30	26.50	73.5
Meat	16.30	6.90	23.20	76.8

In order to simplify the calculation of the energy exchange there exist, besides the above-mentioned standard figures for the physiological calorific value of the organic foodstuffs, also for the carbon of the carbon dioxide, and for the oxygen other standard factors. Thus for 1 gram of meat (dry substance) free from fat and extractives we have the calculated value of 5.44–5.77 Cal. KÖHLER[2] found 5.678 Cal. for 1 gram of ash and fat-free dried-meat substance of the ox and 5.599 Cal. for the horse. According to FRENTZEL and SCHREUER[3] 45.4 Cal. is calculated for 1 gram of nitrogen in fat and ash-free dried-meat fæces (dog), while 6.97 to 7.45 Cal. is calculated for 1 gram of nitrogen in meat-urine. The calorific urine quotient $\frac{\text{Cal.}}{\text{N}}$ seems still, as above given, not to be constant for human beings at least, but is dependent upon the variety of food.

Instead of the direct determination the heat of combustion can also be determined from the elementary composition according to the following principle as suggested by E. VOIT.[4] If we designate the heat of combustion for 1 gram of the substance by Cal. and the quantity of oxygen necessary for the complete combustion of 1 gram of the substance (= oxygen capacity of the substance) by O, then $\frac{\text{Cal.}}{\text{O}} = K$, which is the combustion value for 1 gram of oxygen. The oxygen capacity can be calculated from the elementary composition, and when the value of K is known, the combustion heat of a chemical compound or a known mixture can be readily determined. The value K is nearly constant for substances of the same groups; but also different groups show among themselves only slight deviation for this value. VOIT obtained the following values for a few of the foodstuffs:

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[1] Zeitschr. f. Biologie, 42.

[2] Zeitschr. f. physiol. Chem., 31.

[3] The works of Frentzel and Schreuer may be found in Arch. f. (Anat. u.) Physiol., 1901, 1902, and 1903.

[4] Zeitschr. f. Biologie, 44. See also Krummacher, *ibid.*

	K. (in kg. Cal.)	O Capacity.
Plant protein	3.298	1.740
Animal protein	3.273	1.741
Fat	3.271	2.863
Carbohydrate	3.525	1.156

These methods of calculation are, according to VOIT and KRUMMACHER, admissible for practical purposes.

II. Metabolism in Starvation.

In starvation the decomposition in the body continues uninterruptedly, though with decreased intensity; but, as it takes place at the expense of the substance of the body, it can only continue for a limited time. When an animal has lost a certain fraction of the mass of the body death is the result. This fraction varies with the condition of the body at the beginning of the starvation period. Fat animals succumb when the weight of the body has sunk to one half of the original weight. Otherwise, according to CHOSSAT,[1] animals die as a rule when the weight of the body has sunk to two fifths of the original weight. The period when death occurs from starvation not only varies with the varied nutritive condition at the beginning of starvation, but also with the more or less active exchange of material. This is more active in small and young animals than in large and older ones, but different classes of animals show an unequal activity. Children succumb in starvation in 3–5 days after having lost one fourth of their body mass. Grown persons may, as observed upon SUCCI,[2] and other professional fasters, starve for twenty days or more without lasting injury; and there are reports of cases of starvation extending over a period of even more than forty to fifty days. Dogs can live without food from four to eight weeks, birds five to twenty days, snakes more than half a year, and frogs more than a year.

In starvation the *weight of the body* decreases. The loss of weight is greatest in the first few days, and then decreases rather uniformly. In small animals the absolute loss of weight per day is naturally less than in larger animals. The relative loss of weight — that is, the loss of weight of the unit of the weight of the body, namely, 1 kilo — is, on the contrary, greater in small animals than in larger ones. The reason for this is that the smaller animals have a greater surface of body in proportion to their mass than larger animals, and the greater loss of heat caused thereby must be replaced by a more active consumption of material.

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[1] Cited from Voit in Hermann's Handbuch, **6**, Thl. 1, 100.

[2] See Luciani, Das Hungern. Hamburg u. Leipzig, 1890.

It follows from the decrease in the weight of the body that the absolute extent of metabolism must diminish in starvation. If, on the contrary, the extent of the metabolism is referred to the unit of the weight of the body, namely, 1 kilo, it appears that this quantity remains nearly unchanged during starvation. The investigations of ZUNTZ, LEHMANN, and others[1] on the professional faster CETTI showed on the third and sixth days of starvation an average consumption of 4.65 c.c. oxygen per kilo in one minute, and on the ninth to eleventh day an average of 4.73 c.c. The calories, as a measure of the metabolism, fell on the first to fifth day of starvation from 1850 to 1600 calories, or from 32.4 to 30 per kilo, and it remained nearly unchanged, if referred to the unit of body weight.[2]

The *extent of the metabolism of proteins*, or the elimination of nitrogen by the urine, which is a measure of the same, diminishes as the weight of the body diminishes. This decrease is not regular or the same during the entire period of starvation, and the extent depends, as the experiments made upon carnivora have shown, upon several circumstances. During the first few days of starvation the excretion of nitrogen is greatest, and the richer the body is in protein, due to the food previously taken, the greater is the protein catabolism or the nitrogen elimination, according to VOIT. The nitrogen elimination diminishes the more rapidly — that is, the curve of the decrease is more sudden — the richer in proteins the food was which was taken before starvation. This condition is apparent from the following table of data of three different starvation experiments made by VOIT[3] on the same dog. This dog received 2500 grams of meat daily before the first series of experiments, 1500 grams of meat daily before the second series, and a mixed diet relatively poor in nitrogen before the third series.

Day of Starvation.	**Grams of Urea Eliminated in Twenty-four Hours.** **Ser. I.**	**Ser. II.**	**Ser III**
First	60.1	26.5	13.8
Second	24.9	18.6	11.5
Third	19.1	15.7	10.2
Fourth	17.3	14.9	12.2
Fifth	12.3	14.8	12.1
Sixth	13.3	12.8	12.6
Seventh	12.5	12.9	11.3
Eighth	10.1	12.1	10.7

In man and also in animals sometimes a rise in the nitrogen excretion is observed about the second or third starvation day, which is then followed by a regular diminution. This rise is explained by PRAUSNITZ,

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[1] Berlin. klin. Wochenschr., 1887.

[2] See also Tigerstedt and collaborators in Skand. Arch. f. Physiol., **7**.

[3] See Hermann's Handbuch, **6**, Thl. 1, 89.

Tigerstedt, Landergren,[1] as follows: At the commencement of starvation the protein metabolism is reduced by the glycogen still present in the body. After the consumption of the glycogen, which takes place in great part during the first days of starvation, the destruction of proteins increases as the glycogen action decreases, and then decreases again when the body has become poorer in available proteins.

Other conditions, such as varying quantities of fat in the body, have an influence on the rapidity with which the nitrogen is eliminated during the first days of starvation. After the first few days of starvation the elimination of nitrogen is more uniform. It may diminish gradually and regularly until the death of the animals or there may be a rise in the last days, a so-called premortal increase. Whether the one or the other occurs, depends upon the relationship between the protein and fat content of the body.

Like the destruction of proteins during starvation, the *decomposition of fat* proceeds uninterruptedly, and the greatest part of the calories needed during starvation are supplied by the fats. According to Rubner and Voit the protein catabolism varies only slightly in starving animals at rest and at an average temperature, and forms a constant portion of the total exchange of energy; of the total calories in dogs 10–16 per cent comes from the protein decomposition and 84–90 per cent from the fats. This is at least true for starving animals which had a sufficiently great original fat content. If on account of starvation the animal has become relatively poorer in fat and the fat content of the body has fallen below a certain limit, then in order to supply the calories necessary a larger quantity of protein is destroyed and the premortal increase now occurs (E. Voit[2]).

Since the fat has a diminishing influence on the destruction of proteins corresponding to what was said above, the elimination of nitrogen in starvation is less in fat than in lean individuals. For instance, only 9 grams of urea were voided in twenty-four hours during the later stages of starvation by a well-nourished and fat person suffering from disease of the brain, while I. Munk found that 20–29 grams urea were voided daily by Cetti,[3] who had been poorly nourished.

The investigations on the *exchange of gas* in starvation have shown, as previously mentioned, that the absolute extent of the same is diminished, but that when the consumption of oxygen and elimination of carbon dioxide are calculated on the unit of weight of the body, 1 kilo, this quantity quickly sinks to a minimum and then remains unchanged, or, on the continuation of the starvation, may actually rise. It is a well known fact that the body temperature of starving animals remains nearly constant, without showing any appreciable decrease, during the greater part of the starvation period. The temperature of the animal first sinks a few days before death, and death occurs at about 33–30° C.

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[1] Prausnitz, Zeitschr. f. Biologie, **29**; Tigerstedt and collaborators, l. c.; Landergren, "Undersokningar öfver menniskans ägghviteomsättning, Inaug.-Diss. Stockholm, 1902.

[2] Zeitschr. f. Biologie, **41**, 167 and 502. See also Kaufmann, *ibid.*, and N. Schulz, *ibid.*, and Pflüger's Arch., **76**.

[3] Berl. klin. Wochenschr., 1887.

From what has been said about the respiratory quotient it follows that in starvation it is about the same as with fat and meat exclusively as food, i.e., approximately 0.7. This is often the case, but it may occasionally be lower, 0.65–0.50, as observed in the cases of CETTI and SUCCI. As explanation for this unexpected behavior one must admit of a storage of incompletely oxidized substances in the body during starvation.

Water passes uninterruptedly from the body in starvation even when none is taken. If the quantity of water in the tissues rich in proteins is considered as 70–80 per cent, and the quantity of proteins in the same 20 per cent, then for each gram of protein destroyed about 4 grams of water are set free. This liberation of water from the tissues is generally sufficient to supply the loss of water, and starvation is ordinarily not accompanied with thirst. Starving animals, as a rule, do not partake of water.

The loss of water calculated on the percentage of the total organism must naturally be essentially dependent upon the previous amount of fatty tissue in the body. If we bear these conditions in mind, then, it seems, according to BÖHTLINGK,[1] that, from experiments upon white mice, the animal body is poorer in water during inanition. The body loses more water than is set free by the destruction of the tissues.

The *mineral substances* leave the body uninterruptedly in starvation until death, and the influence of the destruction of tissues is plainly perceptible by their elimination. Because of the destruction of tissues rich in potassium the proportion between potassium and sodium in the urine changes in starvation, so that, contrary to the normal conditions, the potassium is eliminated in proportionately greater quantities. MUNK also observed in CETTI's[2] case a relative increase in the phosphoric acid and calcium in the urine during starvation, which was due to an increased exchange of bone-substance.

Contrary to the above BÖHTLINGK with starving white mice, and KATSUYAMA[3] with starving rabbits found a greater excretion of sodium than potassium.

The question as to the participation of the different organs in the loss of weight of the body during starvation is of special interest. In elucidation of this point we give the following results of Chossat's **experiments** on pigeons, and those of Voit[1] on a male cat. The results are percentages of weight lost from the original weight of the organ.

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[1] Arch. des sciences biol. de St. Pétersbourg, 5.

[2] L. c.

[3] Böhtlingk, l. c.; Katsuyama· Zeitschr. f. physiol. Chem., 26.

	Pigeon (Chossat).	Male Cat (Voit).
Adipose tissue	93 per cent.	97 per cent.
Spleen	71 " "	67 " "
Pancreas	64 " "	17 " "
Liver	52 " "	54 " "
Heart	45 " "	3 " "
Intestine	42 " "	18 " "
Muscles	42 " "	31 " "
Testicles	— " "	40 " "
Skin	33 " "	21 " "
Kidneys	32 " "	26 " "
Lungs	22 " "	18 " "
Bones	17 " "	14 " "
Nervous system	2 " "	3 " "

The total quantity of blood, as well as the quantity of solids contained therein, decreases, as Panum and others[2] have shown, in the same proportion as the weight of the body. The statements in regard to the loss of water by different organs are somewhat contradictory; according to Lukjanow[3] it seems that the various organs act somewhat differently in this respect.

The above-tabulated results cannot serve as a measure of the metabolism in the various organs during starvation. For instance, the nervous system shows only a small loss of weight as compared with the other organs, but from this it must not be concluded that the exchange of material in this system of organs is least active. The condition may be quite different; for one organ may derive its nutriment during starvation from some other organ and exist at its expense. A positive conclusion cannot be drawn in regard to the activity of the metabolism in an organ from the loss of weight of that organ in starvation. Death by starvation is not the result of the death of all the organs of the body, but it depends more likely upon the disturbance in the nutrition of a few less vitally important organs (E. Voit[4]).

In calculating or determining the loss of weight of the organs in starvation the original fat content of the organs must also be considered. With the consideration of the fat content of the organs, determined or estimated in a special way before the starvation period and at the end, E. Voit[5] has found the following loss of weight in the supposed fat-free organs in starvation, namely, muscles 41 per cent, viscera 42 per cent, skin 28 per cent, and skeleton 5 per cent.

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[1] Cited from Voit in Hermann's Handbuch, **6**, Part 1, 96 and 97.

[2] Panum, Virchow's Arch., **29**; London, Arch. d. scienc. biol. de St. Pétersbourg, **4**.

[3] Zeitschr. f. physiol. Chem., **13**.

[4] Zeitschr. f. Biologie, **41**.

[5] *Ibid.*, **46**.

The knowledge of metabolism during starvation is of the greatest importance in the study of nutrition, and it forms to a certain extent the starting-point for the study of metabolism under different physiological and pathological conditions. To answer the question whether the metabolism of a person in a special case is abnormally increased or diminished it is naturally very important to know the average extent of metabolism of a healthy person under the same circumstances, for comparison. This quantity can be called the *starvation requirement*, that is, the extent of metabolism used in absolute bodily rest and inactivity of the intestinal tract. As a measure of this quantity we determine, according to GEPPERT-ZUNTZ, the extent of gaseous exchange, and especially the consumption of oxygen, of a person lying down, best sleeping, in the early morning and at least twelve hours after a light meal not rich in carbohydrates. The gas volume reduced to 0° C. and 760 mm. Hg pressure is calculated on 1 kilo of body weight and for one minute. The results vary between 3 and 4.5 cc. for the consumption of oxygen, and between 2.5 and 3.5 c.c. for the carbon dioxide. As average 3.81 c.c. oxygen and 3.08 c.c. carbon dioxide are usually given.[1]

The extent of protein destruction cannot be determined in transient experiments, and for these reasons only the values found after several days of starvation are useful. In the starvation experiments on CETTI and SUCCI the elimination of nitrogen per kilo on the fifth to the tenth starvation day was 0.150–0.202 gram N. In a recent starvation experiment made by E. and O. FREUND[2] upon SUCCI the nitrogen excretion on the twenty-first day sank to 2.82 grams N. The portion of the urea nitrogen of the total nitrogen sank from 85–89 per cent on the first days of starvation to 73 per cent on the fifteenth day and 56–54 per cent on the day before the last day of starvation. None of the other nitrogenous constituents examined increased to the same extent as the urea decreased. The amount of neutral sulphur rose from 10 to 40 per cent of the total sulphur. In a recent series of investigations upon the faster SUCCI, BRUGSCH[3] found on the twenty-first to the thirtieth day that the urea only amounted to 54–69.4 per cent of the total nitrogen while the quantity of ammonia, because of a high acidosis, rose to 15.4–35.3 per cent. The amino-acid fraction was also above normal.

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[1] See v. Noorden, Lehrbuch der Pathlogie des Stoffwechsel, Berlin, 1906.

[2] Wien. klin. Rundschau, 1901, Nos. 5 and 6.

[3] Zeitschr. f. exp. Path. u. Therap. 1.

III. Metabolism with Inadequate Nutrition.

The food may be quantitatively insufficient, and the final result is absolute inanition. The food may also be qualitatively insufficient or, as we say, inadequate. This occurs when any of the necessary nutritive bodies are absent in the food, while the others occur in sufficient or perhaps even in excessive amounts.

Lack of Water in the Food. The quantity of water in the organism is greatest during fœtal life, and then decreases with increasing age. Naturally, the quantity differs in various organs. The tissue in the body being poorest in water is the enamel, which is almost free, containing only 2 p. m. water, the teeth about 100 p. m., the fatty tissues 60–120 p. m. The bones, with 140–440 p. m., and the cartilage, with 540–740 p. m., are somewhat richer in water, while the muscles, blood, and glands, with 750 to more than 800 p. m., are still richer. The quantity of water is even greater in the animal fluids (see preceding chapter), and the adult body contains in all about 630 p. m. water.[1] If it is borne in mind that two thirds of the animal organism consists of water; that water is of the very greatest importance in the normal, physical composition of the tissues; moreover that all flow of juices, all exchange of substance, all supply of nutrition, all increase or destruction, and all discharge of the products of destruction, are dependent upon the presence of water; and, in addition, that by its evaporation it is an important regulator of the temperature of the body, we perceive that water must be necessary for life. If the loss of water be not replaced by fresh supplies sooner or later, the organism succumbs and death may occur earlier with lack of water than with complete inanition (LANDAUER, NOTHWANG).

If the water is withdrawn for a certain time, as LANDAUER and especially W. STRAUB have shown, it has an accelerating influence upon the decomposition of protein. This increased destruction has, according to LANDAUER, the purpose of replacing a part of the water withheld (by means of the increased metabolism). The deprivation of water for a short time may, according to SPIEGLER,[2] especially in man, cause a diminution in the protein metabolism by means of a reduced protein absorption.

Lack of Mineral Substances in the Food. In a previous chapter attention was called in several instances to the importance of the mineral bodies and also to the occurrence of certain mineral substances in certain amounts in the various organs. The mineral content of the tissues and fluids is not very great as a rule. With the exception of the skeleton, which contains about 220 p. m. mineral bodies (VOLKMANN[1]), the animal fluids or tissues are poor in inorganic constituents, and the quantity of these amounts, as a rule, only to about 10 p. m. Of the total quantity of mineral substances in the organism, the greatest part occurs in the skeleton, 830 p. m., and the next greatest in the muscles, about 100 p. m. (VOLKMANN).

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[1] See Voit in Hermann's Handbuch, **6**, Tl. I, 345.

[2] Landauer, Maly's Jahresber., **24**; Nothwang, Arch. f. Hygiene, **1892**; Straub, Zeitschr. f. Biologie, **37** and **38**; Spiegler, *ibid.*, **40**.

The mineral bodies seem to be partly dissolved in the fluids and partly combined with organic substances. In accordance with this the organism persistently retains, with food poor in salts, a part of the mineral substances, also such as are soluble, as the chlorides. On the burning of the organic substances the mineral bodies combined therewith are set free and may be eliminated. It is also admitted that they in part combine with the new products of the combustion, and in part with organic nutritive bodies poor in salts or nearly salt-free, which are absorbed from the intestinal canal and are thus retained (VOIT, FORSTER[2]).

If this statement is correct, it is possible that a constant supply of mineral substances with the food is not absolutely necessary, and that the amount of inorganic bodies which must be administered is insignificant. The question whether this be so or not has not, especially in man, been sufficiently investigated; but generally we consider the need of mineral substances by man as very small. It may, however, be assumed that man usually takes with his food a considerable excess of mineral substances.

Experiments to determine the action of an insufficient supply of mineral substances with the food in animals have been made by several investigators, especially FORSTER. He observed, on experimenting with dogs and pigeons with food as poor as possible in mineral substances, that a very suggestive disturbance of the functions of the organs, particularly the muscles and the nervous system, appeared, and that death resulted in a short time, earlier in fact than in complete starvation. On observations made upon himself TAYLOR[3] found on partaking less than 0.1 gram salts *per diem* that the chief disturbance occurred in the muscular system.

BUNGE in opposition to these observations of FORSTER's has suggested that the early death in these cases was not caused by the lack of mineral salts, but more likely by the lack of bases necessary to neutralize the sulphuric acid formed in the combustion of the proteins in the organism; these bases must then be taken from the tissues. In accordance with this view. BUNGE and LUNIN[1] also found, in experimenting with mice, that animals which received nearly ash-free food with the addition of sodium carbonate were kept alive twice as long as those which had the same food without the sodium carbonate. Special experiments also show that the carbonate cannot be replaced by an equivalent amount of sodium chloride, and that to all appearances it acts by combining with the acids formed in the body. The addition of alkali carbonate to the otherwise nearly ash-free food may indeed delay death, but cannot prevent it, and even in the presence of the necessary amount of bases death results for lack of mineral substances in the food.

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[1] See Voit in Hermann's Handbuch, 6, Part 1, 353.

[2] Forster, Zeitschr. f. Biologie, 9. See also Voit in Hermann's Handbuch, 6, Part 1, 354. In regard to the occurrence and the behavior of the various mineral constituents of the animal body see the work of Albu and Neuberg, Physiologie and Pathologie des Mineralstoffwechsel, Berlin, 1906.

[3] University of California Publications, Pathol. 1.

In the above series of experiments made by BUNGE the food of the animal consisted of casein, milk-fat, and cane-sugar. While milk alone was an adequate and sufficient food for the animal, BUNGE found that the animal could not be kept alive longer by food consisting of the above constituents of milk and cane-sugar with the addition of all the mineral substances of milk than with the food mentioned in the above experiments with the addition of alkali carbonate. The question whether this result is to be explained by the fact that the mineral bodies of milk are chemically combined with the organic constituents of the same and can be assimilated only in such combinations, or whether it depends on other conditions, BUNGE leaves undecided. These observations, however, show how difficult it is to draw positive conclusions from experiments made thus far with food poor in salts. Further investigations on this subject seem to be necessary.

With an insufficient supply of *chlorides* with the food the elimination of chlorine by the urine decreases constantly, and at last it may stop entirely, while the tissues still persistently retain the chlorides. It has already been stated (Chapter IX) how chloride starvation influences other functions, especially the secretion of gastric juice. If there be a lack of sodium as compared with potassium, or if there be an excess of potassium compounds in any other form than KCl, the potassium combinations are replaced in the organism by NaCl, so that new potassium and sodium compounds are produced which are voided with the urine. The organism becomes poorer in NaCl, which therefore must be taken in greater amounts from the outside (BUNGE). This occurs habitually in herbivora, and in man with vegetable food rich in potash. For human beings, and especially for the poorer classes of people who live chiefly on potatoes and foods rich in potash, common salt is, under these circumstances, not only a condiment, but a necessary addition to the food (BUNGE[2]). On the behavior of chlorides, especially sodium chloride, in the animal body as well as the elimination or the retention of NaCl in diseases we have an abundance of investigations, which may be found in ALBU and NEUBERG's work previously cited.

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[1] Bunge, Lehrbuch der physiol. Chem., 4. Aufl., 97; Lunin, Zeitschr. f. physiol. Chem., **5**.

[2] Zeitschr. f. Biologie **9**.

Lack of Alkali Carbonates or Bases in the Food. The chemical processes in the organism are dependent upon the presence of tissue-fluids of a certain reaction, and this action, which is habitually alkaline towards litmus and neutral towards phenolphthalein, is chiefly due to the presence of alkali carbonates and carbon dioxide. The alkali carbonates are also of great importance not only as a solvent for certain protein bodies and as constituents of certain secretions, such as the pancreatic and intestinal juices, but they are also a means of transportation of the carbon dioxide in the blood. It is therefore easy to understand that a decrease below a certain point in the quantity of alkali carbonate must endanger life. Such a decrease not only occurs with lack of bases in the food which brings about various disturbances and death by a relatively great production of acids through the burning of the proteins, but it also occurs when an animal is given dilute mineral acids for a period. The importance of ammonia as a means of neutralizing the acids produced or introduced into the body as well as the different resistance of man and other animals towards this action of acids has already been discussed in Chapter XV.

Lack of Phosphates and Earths. With the exception of the importance of the alkaline earths as carbonates and more especially as phosphates in the physical composition of certain structures, such as the bones and teeth, their physiological importance is nearly unknown. The importance of calcium for certain enzymotic processes and also the great importance of calcium ions for the functions of the muscles and especially for cell life give an indication of the great importance of the alkaline earths for the animal organism. Very little is known in regard to the need of these earths in adults, and no average results can be given for this. The same is true for the need of phosphates or phosphoric acid, whose great importance is recognized not only for the construction of the bones but also for the functions of the muscles, the nervous system, the glands, the organs of generation, etc. The extent of this need is most difficult to determine as the body shows a strong tendency, when increased amounts of phosphorus are introduced, to retain more than is necessary. The need of phosphates is relatively smaller in adults than in young, developing animals, and in these latter the question of the action of insufficient supply of earthy phosphates and alkaline earths upon the bone tissue is of special interest. In regard to this question we refer to Chapter X and to the cited work of ALBU-NEUBERG.

Another important question is, How far do the phosphates take part in the construction of the phosphorized constituents of the body or to what extent are they necessary? The experiments of **Röhmann** and his pupils[1] with phosphorized (casein, vitellin) and non-phosphorized proteins (edestin) and phosphates show that with the introduction of casein and vitellin a deposition of nitrogen and phosphorus takes place, while with non-phosphorized protein and phosphates this does not seem to occur. The body apparently does not have the power of building up the phosphorized cell constituents necessary for cell life from non-phosphorized proteins and phosphates. On the contrary, according to the observations of several investigators, the lecithins seem to possess this power. As known from the investigations of Miescher, the development of generative organs of the salmon which are very rich in nuclein substances and phosphatides from the muscles which are relatively poor in organic-combined phosphorus seem to indicate a synthesis of phosphorized organic substance from the phosphates. Other investigators, such as v. Wendt,[2] also admit of a synthesis of phosphorized protein substances by the aid of inorganic phosphates.

Lack of Iron. As iron is an integral constituent of hæmoglobin, absolutely necessary for the introduction of oxygen, just so is it an indispensable constituent of food. Iron is a never-failing constituent of the nucleins and nucleoproteids, and herein lies also another reason for the necessity of the introduction of iron. Iron is also of great importance for the action of certain enzymes, the oxidases. In iron starvation iron is continually eliminated, even though in diminished amounts; and with an insufficient supply of iron with the food the formation of hæmoglobin decreases. The formation of hæmoglobin is not only enhanced by the supply of organic iron, but also, according to the general view, by inorganic iron preparations. The various divergent statements on this question have already been given in a previous chapter (on the blood).

In the absence of *protein bodies* in the food the organism must nourish itself by its own protein substances, and with such nutrition it must sooner or later succumb. By the exclusive administration of fat and carbohydrates the consumption of proteins in these cases is very considerably reduced. According to the doctrine of C. Voit, which has been defended by recent investigations of E. Voit and Korkunoff,[3] the protein metabolism is never so low under these conditions as in starvation. According to several investigators, such as Hirschfeld, Kumagawa, Klemperer, Sivén, Landergren,[4] and others, the protein metabolism may indeed, with exclusively fat and carbohydrate diet, be smaller than in complete starvation. Thus LANDERGREN has observed on an adult, healthy man in nitrogen starvation but with sufficient supply of energy (about 40 calories per 1 kilo as carbohydrates and fat) on the fourth starvation day that the nitrogen excretion was not more than 4 grams. On the seventh day, with only carbohydrates, the nitrogen excretion sank to 3.34 grams, which corresponded to 0.047 gram N per kilo of body weight and to 0.29 gram protein.

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[1] See Marcuse, Pflüger's Arch., **67**, and footnote 2, page 719.

[2] Skand. Arch., f. Physiol. **17**.

[3] Zeitschr. f. Biologie, **32**.

[4] Hirschfeld, Virchow's Arch., **114**; Kumagawa, *ibid.*, **116**; Klemperer, Zeitschr. f. klin. Med., **16**; Sivén, Skand. Arch. f. Physiol., **10** and **11**; Landergren, l. c. **11**; footnote 1, page 730. See also Maly's Jahresber., **32**.

The absence of *fats* and *carbohydrates* in the food affect carnivora and herbivora somewhat differently. It is not known whether carnivora can be kept alive for any length of time by food entirely free from fat and carbohydrates.[1] But it has been positively demonstrated that they can be kept alive a long time by feeding exclusively with meat freed as much as possible from visible fat (PFLÜGER[2]). Human beings and herbivora, on the contrary, cannot live for any length of time on such food. On one side they lose the property of digesting and assimilating the necessarily large amounts of meat, and on the other a distaste for large quantities of meat or proteins soon appears.

A question of greater importance is whether it is possible to maintain life in an animal for any length of time with a mixture of simple organic and inorganic foodstuffs. This was not possible in the experiments of BUNGE and LUNIN, cited above. Later investigators, such as HALL and STEINITZ, FALTA and NOEGGERATH, arrived at somewhat better results; and RÖHMANN[3] has arrived at still more conclusive results. He used mice in his experiments, and fed them with a mixture of casein, white of egg, vitellin, potato-starch, wheat-starch, margarine, and salts. With this diet the animals maintained their body weight and brought forth young. These latter could not be raised on artificial food. A better result was obtained by adding some malt to the food. It was also possible to further raise with artificial food to maturity mice which had been formed and born with artificial food. These mice remained somewhat smaller than the normal, and no living young could be obtained from them. If we exclude the fact that the foodstuffs fed were not all simple (white of egg, malt), pure foods it seems as if artificial mixtures of food are sufficient to maintain at least an adult animal for a long time, while it is not quite sufficient for the development of a young animal.

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[1] See Horbaczewski, Maly's Jahresber., **31**, 715.

[2] Pflüger's Arch., **50**.

[3] Hall, Arch. f. (Anat. u.) Physiol., 1896; Steinitz, Über Versuche mit künstlicher Ernährung, Inaug.-Diss., Breslau, 1900; Falta and Noeggerath, Hofmeister; Beiträge, 7; Röhmann, Klin. therap. Wochenschr., 1902, No. 40.

IV. Metabolism with Various Foods.

For the carnivora, as above stated, meat as poor as possible in fat may be a complete and sufficient food. As the proteins moreover take a special place among the organic nutritive bodies by the quantity of nitrogen they contain, it is proper that we first describe the metabolism with an exclusively meat diet.

Metabolism with food rich in proteins, or feeding only with meat as poor in fat as possible.

By an increased supply of proteins their catabolism and the elimination of nitrogen is increased, and this in proportion to the supply of proteins.

If a certain quantity of meat has been given to carnivora as food daily and the quantity is suddenly increased, an augmented catabolism of proteins, or an increase in the quantity of nitrogen eliminated, is the result. If the animal is fed daily for a certain time with larger quantities of the same meat, a part of the proteins accumulates in the body, but this part decreases from day to day, while there is a corresponding daily increase in the elimination of nitrogen. In this way a nitrogenous equilibrium is established; that is, the total quantity of nitrogen eliminated is equal to the quantity of nitrogen in the absorbed proteins or meat. If, on the contrary, an animal which is in nitrogenous equilibrium, having been fed on large quantities of meat, is suddenly given a small quantity of meat per day, then the animal uses up its own body proteins, the amount decreasing from day to day. The elimination of nitrogen and the catabolism of proteins decrease constantly, and the animal may in this case also pass into nitrogenous equilibrium, or nearly into this condition. These relations are illustrated by the following table (Voit[1]):

	Grams of Meat in the Food per Day.	
	Before the Test.	During the Test.
1.	500	1500
2.	1500	1000

Grams of Flesh Metabolized in Body per Day.						
1	2	3	4	5	6	7
1222	1310	1390	1410	1440	1450	1500
1153	1086	1088	1080	1027		

In the first case (1) the metabolism of meat before the beginning of the actual experiment on feeding with 500 grams of meat was 447 grams, and it increased considerably on the first day of the experiment, after feeding with 1500 grams of meat. In the second case (2), in which the animal was previously in nitrogenous equilibrium with 1500 grams of meat, the metabolism of flesh on the first day of the experiment, with only 1000 grams meat, decreased considerably, and on the fifth day nearly a nitrogenous equilibrium was obtained. During this time the animal gave up daily some of its own proteins. Between that point below which the animal loses from its own weight and the maximum, which seems to be dependent upon the digestive and assimilative capacity of the intestinal canal, a carnivora may be kept in nitrogenous equilibrium with varying quantities of proteins in the food.

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[1] Hermann's Handbuch, 6, Part I, 110.

The supply of proteins, as well as the protein condition of the body, affects the extent of the protein metabolism. A body which has become rich in proteins by a previous abundant meat diet must, to prevent a loss of proteins, take up more protein with the food than a body poor in proteins.

In regard to the rapidity with which the protein catabolism takes place FALTA[1] has found in man but not, or at least not to the same extent, in dogs, that quite great differences exist between the different proteins. Thus on feeding pure proteins the chief amount of the nitrogen is much quicker eliminated after feeding casein than after genuine ovalbumin. This latter is much easier demolished after a previous denaturization by coagulation than in the native state, which indicates that an unequal resistance of the different proteins towards the digestive juices plays a part. Even on feeding with easily decomposable proteins it takes always several days before the total nitrogen corresponding thereto is eliminated, which depends according to FALTA upon a progressive demolition of the protein. From the unequal rapidity with which the different proteins are decomposed it follows that in the passage from a diet poor in protein to one rich in protein the time within which nitrogenous equilibrium occurs depends chiefly upon the kind of protein contained in the food.

PETTENKOFER and VOIT have made investigations on the *metabolism of fat* with an exclusively protein diet. These investigations have shown that by increasing the quantity of proteins in the food the daily metabolism of fat decreases, and they have drawn the conclusion from these experiments, as detailed in Chapter X, that even a formation of fat may take place under these circumstances. The objections presented by PFLÜGER to these experiments, as well as the proofs of the formation of fat from proteins, are also given in the above-mentioned chapter.

According to PFLÜGER's doctrine the protein can influence the formation of fat only in an indirect way, namely, in that it is consumed instead of the non-nitrogenous bodies and hence the fat and fat-forming carbohydrates are spared. If sufficient protein is introduced into the food to satisfy the total nutritive requirements, then the decomposition of fat stops; and if non-nitrogenous food is taken at the same time, this is not consumed, but is stored up in the animal body, the fats as such, and the carbohydrates at least in great part as fat.

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[1] Deutsch. Arch. f. klin. Med. 86.

PFLÜGER defines the "nutritive requirement" as the smallest quantity of lean meat which produces nitrogenous equilibrium without causing any decomposition of fat or carbohydrates. At rest and at an average temperature it is found for dogs to be 2.073 to 2.099 grams of nitrogen[1] (in meat fed) per kilo of flesh weight (not body weight, as the fat, which often forms a considerable fraction of the weight of the body, cannot as it were be used as dead measure). Even when the supply of protein is in excess of the nutritive requirements, PFLÜGER has found that the protein metabolism increases with an increased supply until the limit of digestive power is reached, which limit is about 2600 grams of meat with a dog weighing 30 kilos. In these experiments of PFLÜGER's all of the excess of protein introduced was not completely decomposed, but a part was retained by the body. PFLÜGER therefore defends the proposition "that a supply of proteins only, without fat or carbohydrate, does not exclude a protein fattening."

From what has been said on protein metabolism in starvation and with exclusive protein food it follows that the protein catabolism in the animal body never stops, that the extent is dependent in the first place upon the extent of protein supply, and that the animal body has the property, within wide limits, of accommodating the protein catabolism to the protein supply.

These and certain other peculiarities of protein catabolism have led VOIT to the view that all proteins in the body are not decomposed with the same ease. VOIT differentiates the protein fixed in the tissue-elements, so-called organized proteins, *tissue-proteins*, from those proteins which circulate with the fluids in the body and its tissues and which are taken up by the living cells of the tissues from the interstitial fluids washing them and are destroyed. These *circulating proteins* are, according to VOIT, more easily and quickly destroyed than the tissue-proteins. When, therefore, in a fasting animal which has been previously fed with meat an abundant and quickly decreasing decomposition of proteins takes place, while in the further course of starvation this protein catabolism becomes less and more uniform, this depends upon the fact that the supply of circulating proteins is destroyed chiefly in the first days of starvation and the tissue-proteins in the last days.

The tissue-elements constitute an apparatus of a relatively stable nature, which have the power of taking proteins from the fluids washing the tissues and appropriating them, while their own proteins, the tissue-proteins, are ordinarily catabolized to only a small extent, about 1 per cent daily (VOIT). By an increased supply of proteins the activity of the cells and their ability to decompose nutritive proteins is also increased to a certain degree. When nitrogenous equilibrium is obtained after an increased supply of proteins, it denotes that the decomposing power of the cells for proteins has increased so that the same quantity of proteins is metabolized as is supplied to the body. If the protein metabolism is decreased by the simultaneous administration of other non-nitrogenous foods (see below), a part of the circulating proteids may have time to become fixed and organized by the tissues, and in this way the mass of the flesh of the body increases. During starvation or with a lack of proteins in the food the reverse takes place, for a part of the tissue proteins is converted into circulating proteins which are metabolized, and in this case the flesh of the body decreases.

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[1] See Schöndorff, Pflüger's Arch., **71**.

VOIT's theory has been criticised by several investigators and especially by PFLÜGER. PFLÜGER's statement, based on an investigation made by one of his pupils, SCHÖNDORFF,[1] is that the extent of protein destruction is not dependent upon the quantity of circulating proteins, but upon the nutritive condition of the cells for the time being — a view which is not very contradictory of VOIT if the AUTHOR does not misunderstand PFLÜGER. VOIT[2] has, as is known, stated that the conditions for the destruction of substances in the body exist in the cells, and also that the circulating protein, likewise according to VOIT, is first catabolized after having been taken up by the cells from the fluids washing them. The point of VOIT's theory is that all proteins are not destroyed in the body with the same degree of readiness. The organized protein, which is fixed by the cells and has become a part of the same, is destroyed less readily, according to VOIT, than the protein taken up by the cells from the nutritive fluid, which serves as material for the chemical construction of the very much more complicated organized proteins. This nutritive protein, which circulates with the fluids before it is taken up by the cells, and which can exist in store in the cells as well as in the fluids, agreeably to VOIT's view, has been called circulating protein or supply protein by him. It is clear that these names may lead to misunderstanding, and therefore too much stress should not be put upon them. The most essential part of VOIT's theory is the supposition that the food protein of the cells is more easily destroyed than the organized, real protoplasmic protein, and this assertion can hardly, for the present, be considered as refuted or exactly proved.

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[1] Pflüger, Pflüger's Arch., 54; Schöndorff, *ibid.*, 54.

[2] Zeitschr. f. Biologie, 11.

The investigations in recent years, especially those of FOLIN, which show that the amount of certain nitrogenous urinary constituents, such as creatinine, uric acid and the combinations containing neutral sulphur are nearly independent upon the quantity of portein taken as food, while the quantity of urea is determined by the protein partaken of, speaks without any doubt in favor of VOIT's view that we must differentiate between the real cell protein and the food protein. This has also led FOLIN to differentiate between endogenous and exogenous protein metabolism. The experience on protein feeding and the endeavor of the body, as observed by SCHREUER,[1] on going to an ordinary diet after abundant protein feeding, to remain at the old state previous to the over feeding of protein, speak also for the fact that protein retained by the body is not quite the same as the other body protein.

This question is intimately connected with another, namely, whether the food proteins taken up by the cells are metabolized as such or whether they are first organized, i.e., are converted into specific cell protein. The investigations of PANUM and FALCK and others[2] on the transitory progress of the elimination of urea after a meal rich in proteins throws light on this question. From experiments upon a dog it was found that the elimination of urea increases almost immediately after a meal rich in proteins, and that it reaches its maximum in about six hours, when about one half of the quantity of nitrogen corresponding to the administered proteins is eliminated. If we also recollect that, according to an experiment of SCHMIDT-MÜLHEIM[3] upon a dog, about 37 per cent of the given proteins are absorbed in the first two hours after the meal and about 59 per cent in the course of the first six hours, it may then be inferred that the increased elimination of nitrogen after a meal is due to a catabolization of the digested and assimilated proteins of the food not previously organized. If it is admitted that the catabolized protein must have been organized, then the greatly increased elimination of nitrogen after a meal rich in proteins supposes a far more rapid and comprehensive destruction and reconstruction of the tissues than has been generally assumed.

The extensive cleavage of the proteins in digestion and the repeatedly observed deamidation of amino acids in the animal body make it probable that the abundant elimination of nitrogen after a diet rich in protein is in great part due to a progressive demolition of the food protein in digestion whereby certain atomic complexes are more readily split than others. The abundant elimination of nitrogen by the urine after partaking considerable protein may also depend in great part upon these nitrogenous atomic complexes which are split off and whose nitrogen is split off as ammonia and therefore cannot be used by the body. The abundant formation of ammonia in the cells of the digestive apparatus after food rich in proteins, as observed by NENCKI and ZALESKI[1] seem to speak in favor of this view.

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[1] Folin, Amer. Journ. of Physiol., **13**; Schreuer, Pflüger's Arch., **110**.

[2] Panum, Nord. Med. Arkiv., **6**; Falck, see Hermann's Handbuch, **6**, Part I, 107. For further statements in regard to the curve of nitrogen elimination in man, see Tschenloff, Korrespond. Blatt Schweiz. Aerzte, 1896; Rosemann, Pflüger's Arch., **65**, and Veraguth, Journ. of Physiol., **21**; Schlosse, Maly's Jahresber., **31**.

[3] Arch. f. (Anat. u.) Physiol., 1879.

In this connection it must be recalled that, according to the investigations of RIAZANTSEFF, substantiated by SCHEPSKI, after partaking of food an increased nitrogen elimination depends in part upon the increased work of the digestive glands. The observations of RIAZANTSEFF[2] that after so-called "apparent feeding" an increased elimination of nitrogen occurs has not been confirmed by the recent observations of COHNHEIM and therefore cannot be considered as conclusive.

It has been stated above that other foods may decrease the catabolism of proteins. Gelatine is such a food. *Gelatine* and the *gelatine-formers* do not seem to be converted into protein in the body, and this last cannot be entirely replaced by gelatine in the food. For example, if a dog is fed on gelatine and fat, its body sustains a loss of proteins even when the quantity of gelatine is so large that the animal, with an amount of fat and meat containing just the same quantity of nitrogen as the gelatine in question, may remain in nitrogenous equilibrium. On the other hand, gelatine, as VOIT, PANUM, and OERUM[3] have shown, has a great value as a means of sparing the proteins, and it may decrease the catabolism of proteins to a still greater extent than fats and carbohydrates. This is apparent from the following summary of VOIT's experiments upon a dog:

Food per Day.				Flesh.	
Meat.	Gelatine.	Fat.	Sugar.	Catabolized.	On the Body.
400	0	200	0	450	−50
400	0	0	250	439	−39
400	200	0	0	356	+44

I. MUNK[4] has later arrived at similar results by means of more decisive experiments. He found in dogs that on a mixed diet which contained 3.7 grams protein per kilo of body, of which hardly 3.6 grams was catabolized, nearly $\frac{5}{6}$ could be replaced by gelatine. The same dog catabolized on the second day of starvation three times as much protein as with the gelatine feeding. MUNK states also that gelatine has a much greater sparing action on proteins than the fats or the carbohydrates.

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[1] Arch. des scienc. biol. de St. Pétersbourg 4; Salaskin, Zeitschr. f. physiol. Chem. **25**; Nencki and Zaleski, Arch. f. exp. Path. u. Pharm. **37**.

[2] Arch. des scienc. biol. de St. Pétersbourg, 4, 393; Schepski, Maly's Jahresber., **30**; Cohnheim, Zeitschr. f. physiol., Chem. **46**.

[3] Voit, l. c., 123; Panum and Oerum, Nord. Med. Arkiv., **11**.

[4] Pflüger's Arch., **58**.

This ability of gelatine to spare the proteins is explained by VOIT by the fact that the gelatine is decomposed instead of a part of the circulating proteins, whereby a part of this last may be organized.

The recent investigations of KRUMMACHER and KIRCHMANN show the extent of the sparing action of gelatine upon proteins. The extent of protein destruction during gelatine feeding was compared with the extent of protein catabolism in starvation, and it was found that 35–37.5 per cent of the quantity of protein decomposed in starvation could be spared by gelatine. The physiological availability of gelatine was found by KRUMMACHER to be equal to 3.88 calories for 1 gram, which corresponds to about 72.4 per cent of the energy-content of the gelatine. KAUFMANN,[1] who experimented upon dogs, found that ⅕ of the protein nitrogen could be readily replaced by gelatine nitrogen, while in an experiment upon himself with 93 per cent gelatine nitrogen, 4 per cent tyrosine nitrogen, 2 per cent cystin nitrogen, and 1 per cent tryptophane nitrogen, he found instead of the equal quantity of protein nitrogen in the periods before and after, that the gelatine replaced by amino acids had nearly the same physiological value as the proteins.

Gelatine may also decrease somewhat the consumption of fat, although it is of less value in this respect than the carbohydrates.

The question of the nutritive value of proteoses (and peptones) stands in close relationship to the nutritive value of the proteins and gelatine. The early investigations made by MALY, PLÓSZ and GYERGYAY, and ADAMKIEWICZ have led to the conclusion that with food which contains no proteins besides peptones (proteoses) an animal may not only preserve its nitrogenous equilibrium, but its protein condition may even increase. According to recent and more exact investigations by POLLITZER, ZUNTZ, and MUNK the proteoses have the same nutritive value as proteins, at least in short experiments. According to POLLITZER this is true for different proteoses as well as for true peptone; but this does not correspond with the experience of ELLINGER,[2] who finds that the true antipeptone (gland peptone) is not able to entirely replace proteins or to prevent the loss of protein in the animal body. On the contrary, according to him, it has, like gelatine, the property of sparing proteins. Voit long ago expressed a similar view. According to him the proteoses and peptone may indeed replace the proteins for a short time, but not permanently; they can spare the proteins, but cannot be converted into proteins. According to the researches of Blum[1] the different proteoses have various nutritive values. In his experiments the heteroproteose from fibrin could not replace the proteins of the food, while casein protoproteose had this property.

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[1] Krummacher, Zeitschr. f. Biologie, **42**; Kirchmann, *ibid.*, **40**; Kaufmann, Pflüger's Arch., **109**.

[2] Maly, Pflüger's Arch., **9**; Plosz and Gyergyay, *ibid.*, **10**; Adamkiewicz, "Die Natur und der Nährwerth des Peptons" (Berlin, 1877); Pollitzer, Pflüger's Arch., **37**, 301; Zuntz, *ibid.*, **37**, 313; Munk, Centralbl. f. d. med. Wissensch., 1889, 20, and Deutsch. med. Wochenschr., 1889; Ellinger, Zeitschr. f. Biologie, **33** (literature).

The question as to the nutritive value of proteoses and peptones has turned in a new direction, due to the more recent views, as mentioned in Chapter IX, on the absorption of proteins where the proteins are not absorbed chiefly as proteoses and peptones, but as simpler cleavage products. From these simple products as mentioned in a previous chapter (IX on absorption) a synthesis of protein can take place in the body. Even if such a synthesis takes place and if it were possible to nourish the body for a long time with a mixture of digestion products still it does not follow that proteoses and peptones can completely replace the proteins of the food. The proteoses and peptones are formed by cleagages, and perhaps certain atomic complexes are absent which occur in the mixture of cleavage products and which are necessary for a regeneration of special protein bodies.

We have a number of investigations[2] upon the value of *asparagin*, and the results are still not conclusive so that quite positive deductions can be drawn from them. The experiments upon herbivora seem to indicate that the asparagin has hardly any action upon the deposition of protein while it can have an indirect protein sparing action and may serve in producing temperature. The protein sparing action seems, at least in part, to be explained by its excelerating action upon digestion. In carnivora (I. Munk) and in mice (Voit and Politis) it was found that asparagin has only a very slight, if any, sparing action on the proteins. It is not known how it acts in man.

Metabolism on a Diet consisting of Protein, with Fat or Carbohydrates. Fat cannot arrest or prevent the *catabolism of proteins;* but it can decrease it, and so spare the proteins. This is apparent from the following table of Voit.[3] A is the average for three days, and B for six days.

	Food.		Flesh.	
	Meat.	Fat.	Metabolized.	On the Body.
A	1500	0	1512	−12
B	1500	150	1474	+26

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[1] Zeitschr. f. physiol. Chem., **30**; Voit, l. c., 394.

[2] Weiske, Zeitschr. f. Biologie, **15** and **17**, and Centralbl. f. d. med. Wissensch., 1890, 945; Munk, Virchow's Arch., **94** and **98**; Politis, Zeitschr. f. Biologie, **28**. See also Mauthner, *ibid.*, 28; Gabriel, *ibid.*, **29**; and Voit, *ibid.*, **29**, 125; Kellner, Maly's Jahresber, **27**, and Zeitschr. f. Biologie, **39**; Kellner and Köhler, Chem. Centralbl, **1**, 1906. Völtz Pflüger's Arch. 107; v. Strusiewicz, Zeitschr. f. Biologie 47.

[3] Voit in Hermann's Handbuch **6**, 130.

According to VOIT the adipose tissue of the body acts like the food-fat, and the protein-sparing effect of the former may be added to that of the latter, so that a body rich in fat may not only remain in nitrogenous equilibrium, but may even add to the store of body proteins, while in a lean body with the same food containing the same amount of proteins and fat there would be a loss of proteins. In a body rich in fat a greater quantity of proteins is protected from metabolism by a certain quantity of fat than in a lean body.

Because of the sparing action of fats an animal to whose food fat is added may, as is apparent from the table, increase its store of protein with a quantity of meat which is insufficient to preserve nitrogenous equilibrium.

Like the fats the carbohydrates have a sparing action on the proteins. By the addition of carbohydrates to the food the carnivora not only remains in nitrogenous equilibrium, but the same quantity of meat which in itself is insufficient and which without carbohydrates would cause a loss of weight in the body may with the addition of carbohydrates produce a deposit of proteins. This is apparent from the following table:[1]

Food.				Flesh.	
Meat.	Fat.	Sugar.	Starch.	Metabolized.	On the Body.
500	250	...	...	558	− 58
500	...	300	...	466	+ 34
500	...	200	...	505	− 5
800	...	...	250	745	+ 55
800	200	...	...	773	+ 27
2000	...	...	200–300	1792	+208
2000	250	...	...	1883	+117

The sparing of protein by carbohydrates is greater, as shown by the table, than by fats. According to VOIT the first is on an average 9 per cent and the other 7 per cent of the administered protein without a previous addition of non-nitrogenous bodies. Increasing quantities of carbohydrates in the food decrease the protein metabolism more regularly and constantly than increasing quantities of fat. ATWATER and BENEDICT[2] also found that the carbohydrates had a somewhat greater sparing action upon proteins than fats.

Because of this great protein-sparing action of carbohydrates the herbivora, which as a rule partake of considerable quantities of carbohydrates, assimilate proteins readily (VOIT).

The greater protein-sparing action of carbohydrates as compared to that of the fats occurs, as shown by LANDERGREN,[3] to a still higher degree with food poor in nitrogen or in nitrogen starvation, in which cases the carbohydrates have double the protein-sparing action as compared to an isodynamic quantity of fat.

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[1] Voit, *ibid.*, page 143.

[2] See Ergebnisse der Physiologie **3**.

[3] L. c., Inaug.-Diss., and Skand. Arch. f. Physiol., **14**.

The protein-sparing action of the carbohydrates and fats has generally been studied by the one-sided feeding with one or the other of these two groups of foodstuffs. The question may be raised whether the difference observed between the fats and carbohydrates could not be brought about also by the simultaneous supply of carbohydrates and fat in varying proportions. TALLQUIST[1] has made a series of experiments on this subject. In one of the periods 16.27 grams N, 44 grams fat, and 466 grams carbohydrate were given; in a second, 16.08 grams N, 140 grams fat, and 250 grams carbohydrate, containing nearly the same number of calories, namely, 2867 and 2873 calories. In both cases nearly a complete nitrogenous equilibrium was reached and the carbohydrate did not spare more protein than the fat. It is therefore possible that the fat has about the same protein-sparing action as an isodynamic amount of carbohydrate when the quantity of carbohydrates does not sink below a certain minimum, which is not known for the present.

This condition as well as the extent of protein-sparing action of the carbohydrates stands, according to LANDERGREN,[2] in close relation to the formation of sugar in the body. The animal body always needs sugar, and a lack of carbohydrates in the food leads to a part of the proteins being used in the sugar formation. This part can be spared by carbohydrates but not by fats, from which, according to LANDERGREN, the carbohydrates cannot be formed. In this lies also the probable reason why the fats, on being fed exclusively but not with a sufficient supply of carbohydrates, have a much lower protein-sparing action than the carbohydrates. The fats cannot prevent the protein catabolism necessary for the formation of sugar on a diet lacking in carbohydrates.

The law as to the increased protein catabolism with increased protein supply applies also to food consisting of protein with fat and carbohydrates. In these cases the body tries to adapt its protein catabolism to the supply; and when the daily calorie-supply is completely covered by the food, the organism can, within wide limits, be in nitrogenous equilibrium with different quantities of protein.

The upper limit to the possible protein catabolism per kilo and per day has only been determined for herbivora. For human beings it is not known, and its determination is from a practical standpoint of secondary importance. What is more important is to ascertain the lower limit, and on this subject we have several experiments upon man as well as upon dogs by Hirschfeld, Kumagawa, Klemperer, Munk, Rosenheim,[1] and others. It follows from these experiments that the lower limit of protein needed for human beings for a week or less is about 30–40 grams or 0.4–0.6 gram per kilo with a body of average weight. v. Noorden [2] considers 0.6 gram protein (absorbed protein) per kilo and per day as the lower limit. The above-mentioned figures are only valid for short series of experiments; still there exist the observations of E. Voit and Constantinidi[3] on the diet of a vegetarian when the protein condition was kept nearly normal but not completely for a long time with about 0.6 gram of protein per kilo. Caspari [4] has also made observations upon a vegetarian and for a period of 14 days with an average of 0.1 gram nitrogen (recalculated as equal to 0.62 gram protein) per kilo where a nearly complete nitrogenous equilibrium was observed as the average result.

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[1] Finska Lakaresällskapets handl., 1901. See also Arch. f. Hygiene, 41.

[2] L. c., Inaug.-Diss. See also Skand. Arch. f. Physiol., 14.

According to Voit's normal figures, which will be spoken of below, for the nutritive need of man an average working man of about 70 kilos weight requires on a mixed diet about 40 calories per kilo (true calories or net calories). In the above experiments with food very poor in protein the demand for calories was considerably greater; as, for instance, in certain cases it was 51 (Kumagawa) or even 78.5 calories (Klemperer). It therefore seems as if the above very low supply of protein was only possible with great waste of non-nitrogenous food; but in opposition to this it must be recalled that in Voit and Constantinidi's experiments upon the vegetarian, who for years was accustomed to a food very poor in protein and rich in carbohydrate, the calories amounted to only 43.7 per kilo. In the case studied by Caspari a supply of 41 calories per kilo was entirely sufficient.

Sivén has shown by experiments upon himself that the adult human organism, at least for a short time, can be maintained in nitrogenous equilibrium with a specially low supply of nitrogen without increasing the calories in the food above the normal. With a supply of 41–43 calories per kilo he remained in nitrogenous equilibrium for four days with a supply of nitrogen of 0.08 gram per kilo of body weight. Of the nitrogen taken, a part was of a non-protein nature and the quantity of true protein nitrogen was only 0.045 gram, corresponding to about 0.3 gram of protein per kilo of body weight. That this low limit, which by the way only holds for a short time, has no general validity follows from other observations. Thus Caspari [5] also, in an experiment on himself, could not attain complete nitrogenous equilibrium on a much greater nitrogen supply. The protein minimum seems also to be different for various individuals.

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[1] See footnote 4, page 738; also Munk, Arch. f. (Anat. u.) Physiol., 1891 and 1896; Rosenheim, *ibid.*, 1891; Pflüger's Arch., **54**.

[2] Grundriss einer Methodik der Stoffwechseluntersuchungen. Berlin, 1892.

[3] Zeitschr. f. Biologie, **25**.

[4] Physiologische Studien über Vegetarismus, Bonn, 1905.

[5] Sivén, Skand. Arch. f. Physiol., **10** and **11**; Caspari, Arch. f. (Anat. u.) Physiol. 1901.

The very important question as to the conditions favoring the deposition of fat and flesh in the body is closely associated with what has just been said in regard to foods consisting of protein and non-nitrogenous foodstuffs. In this connection it must be remembered in the first place that all fattening presupposes an overfeeding, i.e., a supply of foodstuffs which is greater than that catabolized in the same time.

In carnivora a flesh deposition may take place on the exclusive feeding with meat. This is not generally large in proportion to the quantity of protein catabolized. As shown by an experiment upon a male cat by Pflüger [1] this may be so great that the body doubles in weight under favorable conditions. In man and herbivora, on the contrary, the demand for calories may not be covered by protein alone, and the question as to the conditions of fattening with a mixed diet is of importance.

These conditions have also been studied in carnivora, and here, as Voit has shown, the relationship between protein and fat (and carbohydrates) is of great importance. If much fat is given in proportion to the protein of the food, as with average quantities of meat with considerable addition of fat, then nitrogenous equilibrium is only slowly attained and the daily deposit of flesh, though not large, is quite constant, and may become greater in the course of time. If, on the contrary, much meat besides proportionately little fat is given, then the deposit of protein with increased catabolism is smaller day by day, and nitrogenous equilibrium is attained in a few days. In spite of the somewhat larger deposit *per diem*, the total flesh deposit is not considerable in these cases. The following experiment of Voit may serve as example:

Number of Days of Experimentation.	Food.		Total Deposit of Flesh.	Daily Deposit of Flesh.	Nitrogenous Equilibrium.
	Meat, Grams.	Fat, Grams.			
32	500	250	1792	56	Not attained
7	1800	250	854	122	Attained

The greatest absolute deposition of flesh in the body was obtained in these cases with only 500 grams of meat and 250 grams of fat, and even after 32 days nitrogenous equilibrium had not occurred. On feeding with 1800 grams of meat and 250 grams of fat nitrogenous equilibrium was established after seven days; and though the deposition of flesh per day was greater, still the absolute deposit was not one half as great as in the former case.

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[1] Pflüger's Arch., 77.

The experiments of KRUG upon himself, under the direction of v. NOORDEN, give us information as to the practicability of flesh deposition in man. With abundant food (2590 cal. = 44 cal. per kilo) KRUG was close to nitrogenous equilibrium for six days. He then increased the nutritive supply to 4300 cal. = 71 cal. per kilo for fifteen days by the addition of fat and carbohydrate, and in this time 309 grams of protein, corresponding to 1455 grams of muscle, was spared. Of the excess of administered calories in this case only 5 per cent was used for flesh deposit and 95 per cent for fat deposit. On the other hand BORNSTEIN,[1] also experimenting upon himself, without any considerable increase in calories, could produce, an increase in his protein condition by about 100 grams of protein, corresponding to 500 grams of flesh, in the course of fourteen days simply by increasing the supply of protein (50 grams of nutrose = sodium casein with 7 grams N per day).

BORNSTEIN arrived at still better results in regard to protein retention by simultaneous muscle work, as in these cases the nitrogen retention corresponded to a flesh deposit of 800 grams. The importance of work for the so-called protein deposition follows also from many other observations, and it is in agreement with daily experience that a man cannot be made muscle-strong by over-feeding alone. A work-hypertrophy must also be introduced.

BORNSTEIN and SCHREUER[2] have given further proof for the possibility of a protein deposition in man and animals (dogs) and there is no doubt that the body becomes richer in active cell masses after abundant supply of protein. This increase seems still, according to SCHREUER, not to be continuous, and the question to what extent the nitrogen retention in so-called protein overfeeding in full-grown animals and man is to be considered as a true flesh enrichment i.e., a new formation of living tissue, seems to require further proof.

The conditions in young, growing individuals are different than in adults. In the first the protein is necessary for the building up of the growing tissue and in them an abundant true flesh deposition takes place. For this protein fattening the amount of supply does not take first place but rather the energy of development. The growing body of the nursling also uses according to RUBNER and HEUBNER,[3] the protein of the food essentially to replace the quantity of protein catabolized and for deposition.

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[1] Krug, cited from v. Noorden, Lehrbuch der Pathologie des Stoffwechsel., 2 Aufl. 557; Bornstein Berl. klin. Wochenschr., 1898, and Pflüger's Arch,. **83** and **106**.

[2] Pflüger's Arch., **110**.

[3] Zeitschr. f. exp. Path. u. Therap. **1**.

It is difficult to produce a permanent flesh deposit in man by overfeeding alone. Flesh deposition is, according to v. NOORDEN, a function of the specific energy of the developing cells and the cell-work to a much higher extent than the excess of food. Therefore there is observed, according to v. NOORDEN, abundant flesh deposition (1) in each growing body; (2) in those no longer growing but whose body is accustomed to increased work; (3) whenever, by previous insufficient food or by disease, the flesh condition of the body has been diminished and therefore requires abundant food to replace the same. The deposition of flesh is in this case an expression of the regenerative energy of the cells.[1]

The experiences of graziers show that in food-animals a flesh deposit does not occur, or at least is only inconsiderable, on overfeeding. The individuality and the race of the animal are of importance for flesh deposition.

As above stated (Chapter X), respecting the formation of fat in the animal body, the most essential condition for a fat deposition is an overfeeding with non-nitrogenous foods. The extent of fat deposition is determined by the excess of calories administered over those actually needed. If a large part of the calorie-demand is covered by protein, then a greater part of the non-nitrogenous foodstuffs simultaneously ingested is spared, i.e., used for fat deposition. But as protein and fat are expensive nutritive bodies as compared with carbohydrates, the supply of greater quantities of carbohydrates is important for fat deposition. The body decomposes less substance at rest than during activity. Bodily rest, besides a proper combination of the three chief groups of organic foods, is therefore also an essential requisite for an abundant fat deposit.

Action of Certain Other Bodies on Metabolism. *Water.* If a quantity in excess of that which is necessary is introduced into the organism, the excess is quickly and principally eliminated with the urine. This increased elimination of urine causes in fasting animals (VOIT, FORSTER), but not to any appreciable degree in animals taking food (SEEGEN, SALKOWSKI and MUNK, MAYER, DUBELIR[2]), an increased elimination of nitrogen. The reason for this increased nitrogen excretion is to be found in the fact that the drinking of much water causes a complete washing out of the urea from the tissues. Another view, which is defended by VOIT, is that because of the more active current of fluids, after taking large quantities of water an increased metabolism of proteins takes place. VOIT considers this explanation the correct one, although he does not deny that by the liberal administration of water a more complete washing out of the urea from the tissues takes place. The views on this question are still somewhat contradictory.[1]

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[1] See also Svenson, Zeitschr. f. klin. Med., **43**.

[2] Voit. Untersuch, über den Einfluss des Kochsalzes, etc. (München, 1860); Forster, cited from Voit in Hermann's Handbuch, **6**, 153; Seegen, Wien. Sitzungsber., **63**; Salkowski and Munk, Virchow's Arch., **71**; Mayer, Zeitschr. f. klin. Med., **2**; Dubelir-Zeitschr. f. Biologie, **28**.

When the body has lost a certain amount of water, then the abstinence from water (in animals) is accompanied by a rise in the protein metabolism (LANDAUER, STRAUB[2]). In regard to the action of water on the formation of fat and its metabolism, the view that the free drinking of water is favorable for the deposition of fat seems to be generally admitted, while the drinking of only very little water acts against its formation.

Salts. The statements are somewhat contradictory in regard to the action of salts, for example sodium chloride and the neutral salts, which partly depends upon the use of large and varying amounts of salt in the experiments. Recent investigations of STRAUB and ROST[3] have shown that the action of salts stands in close relationship to their power of abstracting water. Small amounts of salt which do not produce diuresis have no action on metabolism. On the contrary, larger amounts which bring about a diuresis which is not compensated by the ingestion of water, produce a rise in the protein metabolism. If the diuresis is compensated by drinking water, then the protein metabolism is not increased by salts, but is diminished to a slight degree. An increased nitrogen excretion caused by taking salts can be somewhat increased by the ingestion of water and thus increasing the diuresis, and the action of salts seems to bear a close relationship to the demand and supply of water.

Alcohol. The question as to how far the alcohol absorbed in the intestinal canal is burnt in the body, or whether it leaves the body unchanged by various channels, has been the subject of much discussion. To all appearances the greatest part of the alcohol introduced (95 per cent or more) is burnt in the body (STUBBOTIN, THUDICHUM, BODLÄNDER, BENEDICENTI[4]). As the alcohol has a high calorific value (1 gram = 7 calories), then the question arises whether it acts sparingly on other bodies, and whether it is to be considered as a nutritive substance. The older investigations made to decide this question have led to no decisive result. The thorough investigations of ATWATER and BENEDICT, ZUNTZ and GEPPERT,

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[1] See R. Neumann, Arch. f. Hygiene, **36**; Heilner, Zeitschr. f. Biologie, **47**; Hawk, University of Pennsylvania Med. Bull., xviii.

[2] Landauer, Maly's Jahresber., **24**; Straub, Zeitschr. f. Biologie, **37**.

[3] W. Straub, Zeitschr. f. Biologie, **37** and **38**; Rost, Arbeiten aus d. Kaiserliche Gesundheitsamte, **18** (literature). See also Grüber, Maly's Jahresber., **30**, 612.

[4] Arch. f. (Anat. u.) Physiol., 1896, which contains the literature.

Bjerre, Clopatt, Neumann, Offer, Rosemann,[1] and others, seem to show positively that in man alcohol can diminish the consumption not only of fat and carbohydrates, but also the proteins, although at first, due to its poisonous properties, it may increase the protein metabolism for a short time. The nutritive value of alcohol can only be of special importance in certain cases, as large amounts of alcohol taken at one time, or the continued use of smaller quantities, has an injurious action on the organism. Alcohol may therefore be regarded as a foodstuff only in exceptional cases, and in other respects must be considered as an article of luxury.

Coffee and *tea* have no action on the exchange of material which can be positively proved, and their importance lies chiefly in their action upon the nervous system. It is impossible to enter into the effect of various therapeutic agents upon metabolism.

V. The Dependence of Metabolism on Other Conditions.

The so-called starvation requirement which was previously mentioned, i.e., the extent of metabolism with absolute rest of body and in activity of the intestinal tract, serves best as a starting-point for the study of metabolism under various external circumstances. The metabolism going on under these conditions leads in the first place to the production of heat, and it is only to a subordinate degree dependent upon the work of the circulatory and respiratory apparatus and the activity of the glands. According to a calculation by Zuntz,[2] only 10–20 per cent of the total calories of the starvation requirement belongs to the circulation and respiration work.

The magnitude of the starvation requirement depends in the first place upon the heat production necessary to cover the loss of heat, and this heat production is in turn dependent upon the relationship between the weight and the surface of the body.

Weight of Body and Age. The greater the mass of the body the greater the absolute consumption of material; while, on the contrary, other things being equal, a small individual of the same species of animal metabolizes absolutely less, but relatively more as compared with the unit of the weight of the body. It must be remarked that the relation between flesh and fat in the body exerts an important influence. The extent of the metabolism is dependent upon the quantity of active cells, and a very fat individual therefore decomposes less substance per kilo than a lean person of the same weight. According to RUBNER[1] the importance of the size of the flesh or cell-mass in the body is overestimated. In his investigations on two boys, one of whom was corpulent and the other normally developed, and on comparing the food-need with that found by CAMERER for boys of the same weight, RUBNER came to the result that the exchange of force in the corpulent boy almost completely corresponded with that in the non-corpulent boy of the same weight. By approximately estimating the quantity of fat in the body RUBNER was also able, from the protein condition, to compare the calculated exchange of energy with that actually found. The exchange per kilo amounted to 52 calories in the lean and 43.6 cal. in the fat boy, while, if the protein condition was a measure, one would expect an exchange of calories of only 35 cal. for the fat person. We cannot therefore admit of a diminished activity of the cell-mass in the fat boy, but rather an increased activity. According to RUBNER it is not the flesh-mass (protein mass) alone, but its variable functional changes, which determines the extent of decomposition. In women, who generally have less body weight and a greater quantity of fat than men, the metabolism in general is smaller, and the latter is ordinarily about four fifths that of men.

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[1] In regard to the literature on this subject, see the works of O. Neumann, Arch. f. Hygiene, 36 and 41, and Rosemann, Pflüger's Arch., 86 and 94. A summary of the entire literature upon alcohol can be formed in Abderhalden, "Bibliographie der gesamten wissenschaftlichen Literature über den Alcohol und den Alcoholismus," Berlin and Wien, 1904.

[2] Cited from v. Noorden's Handbuch. 2 Aufl.

The question as to what extent *sex* specially influences metabolism remains to be investigated. TIGERSTEDT and SONDÉN[2] found that in the young the carbon-dioxide elimination, per kilo of body weight as well as per square meter of body surface, was considerably greater in males than in females of the same age and the same weight of body. This difference between the two sexes seems to disappear gradually, and at old age it is entirely absent.[3]

The essential reason why small animals catabolize relatively more substance than large ones, when calculated per kilo body weight, is that the bodies of smaller animals have greater surface in proportion to their mass. On this account the loss of heat is greater, which causes increased heat production, i.e., a more active metabolism. This is also the reason why young individuals of the same kind show a relatively greater metabolism than older ones. If the heat production and carbon-dioxide elimination is calculated on the unit of surface of the body, we find, on the contrary, as the experiments of RUBNER, RICHET,[4] and others show, that they vary only slightly from a certain average in individuals of different weights.

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[1] Beitrage zur Ernahrung im Knabenalter, etc. Berlin, 1902

[2] Skand. Arch. f. Physiol., **6**.

[3] In regard to metabolism and its relationship to the phases of sexual life and especially under the influence of menstruation and pregnancy, see the investigations of A Ver Eecke (Bull. acad. roy. de méd. de Belgique, 1897 and 1901, and Maly's Jahresber., **30** and **31**).

[4] Rubner, Zeitschr. f. Biologie, **19** and **21**; Richet, Arch. de Physiol, 5. **(2)**.

According to RUBNER's rule as to the influence of the surface, which has been recently formulated by E. VOIT, the need of energy in homoiothermic animals is influenced by the development of their surface when their body is given rest, medium surrounding temperature, and relatively equal protein condition. This rule not only applies to adult human beings but also to children and growing individuals (RUBNER, OPPENHEIMER). The surface is the essential factor in determining the extent of exchange of energy. In order to show this we will give here, from a work of RUBNER,[1] the figures representing the quantity of heat in calories for 1 square meter of surface for twenty-four hours.

Adult, medium diet, rest	1189	Calories.
Adult, medium diet, work	1399	"
Suckling	1221	"
Child with medium diet	1447	"
Aged men and women	1099	"
Women	1004	"

The variation in the calorific values[2] found by many investigators, which is sometimes not very small, speaks for the fact that the surface rule is not alone decisive for the exchange of material in resting animals. Still it is generally considered that it is of the greatest importance for metabolism.

The more active metabolism in young individuals is apparent when we measure the gaseous exchange as well as the excretion of nitrogen. As example of the elimination of urea in children the following results of CAMERER[3] are of value:

Age.	Weight of Body in Kilos.	Urea in Grams.	
		Per Day.	Per Kilo.
1½ years	10.80	12.10	1.35
3 "	13.30	11.10	0.90
5 "	16.20	12.37	0.76
7 "	18.80	14.05	0.75
9 "	25.10	17.27	0.69
12½ "	32.60	17.79	0.54
15 "	35.70	17.78	0.50

In adults weighing about 70 kilos, from 30 to 35 grams of urea per day are eliminated, or 0.5 gram per kilo. At about fifteen years of age the destruction of proteins per kilo is about the same as in adults. The relatively greater metabolism of proteins in young individuals is explained partly by the fact that the metabolism of material in general is more active in young animals, and partly by the fact that young animals are, as a rule, poorer in fat than those full grown.

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[1] Rubner, Ernährung im Knabenalter, page 45; E. Voit, Zeitschr. f. Biologie, 41; Oppenheimer, *ibid.*, 42.

[2] See Magnus-Levy, Pflüger's Arch., 55; Slowtzoff (u. Zuntz), *ibid.*, 96.

[3] Zeitschr. f. Biologie, 16 and 20.

According to TIGERSTEDT and SONDÉN the greater metabolism in young animals depends nevertheless also in part on the fact that in these individuals the decomposition in itself is more active than in older ones. The period of growth has a considerable influence on the extent of metabolism (in man), and indeed the metabolism, even when calculated on the unit of surface of body, is greater in youth than in old age. This view is strongly disputed by RUBNER. He does not deny that differences exist between young and adult individuals which may be considered as a deviation from the above rule; still these differences may, according to RUBNER, be dependent upon the work performed, the food, and the nutritive condition. MAGNUS-LEVY and FALK[1] have reported observations which support the views of SONDÉN and TIGERSTEDT.

In old age the metabolism is very much reduced; and even when calculated upon the square meter of surface of body it is lower than in an individual of medium age.

As the metabolism may be kept at its lowest point by absolute rest of body and inactivity of the intestinal tract, it is manifest that work and the ingestion of food have an important bearing on the extent of metabolism.

Rest and Work. During work a greater quantity of chemical energy is converted into kinetic energy, i.e., the metabolism is increased more or less on account of work.

As explained in a previous chapter (XI), work, according to the generally accepted view, has no material influence on the excretion of nitrogen. It is nevertheless true that several investigators have observed in certain cases an increased elimination of nitrogen; but these observations have been explained in other ways. For instance, work may, when it is connected with violent movements of the body, easily cause dyspnœa, and this last, as FRÄNKEL[2] has shown, may occasion an increase in the elimination of nitrogen, since diminution of the oxygen supply increases the protein metabolism. In other series of experiments the quantity of carbohydrates and fats in the food was not sufficient; the supply of fat in the body was decreased thereby, and the destruction of proteins was correspondingly increased. Other conditions, such as the external temperature and the weather,[3] thirst, and drinking of water, can also influence the excretion of nitrogen. According to the generally accepted views muscular activity has hardly any influence on the metabolism of proteins.

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[1] Tigerstedt and Sondén, l. c.; Rubner, l. c.; Magnus-Levy, Arch. f. (Anat. u.) Physiol., 1899, Suppl.

[2] Virchow's Arch., 67 and 71.

[3] See Zuntz and Schumburg, Arch. f. (Anat. u.) Physiol., 1895.

On the contrary, work has a very considerable influence on the elimination of carbon dioxide and the consumption of oxygen. This action, which was first observed by LAVOISIER, has later been confirmed by many investigators. PETTENKOFER and VOIT[1] have made investigations on a full-grown man as to the metabolism of the nitrogenous as well as of the non-nitrogenous bodies during rest and work, partly while fasting and partly on a mixed diet. The experiments were made on a full-grown man weighing 70 kilos. The results are contained in the following table:

		Consumption of				
		Proteins.	Fat.	Carbohydrates.	CO$_2$ Eliminated.	O Consumed.
Fasting ...	Rest......	79	209	...	716	761
	Work.....	75	380	...	1187	1071
Mixed diet	Rest......	137	72	352	912	831
	Work.....	137	173	352	1209	980

In these cases work did not seem to have any influence on the destruction of proteins, while the gas exchange was considerably increased.

ZUNTZ and his pupils[2] have made very important investigations into the extent of the exchange of gas as a measure of metabolism during work and caused by work. These investigations not only show the important influence of muscular work on the catabolism of material, but they also indicate in a very instructive way the relationship between the extent of metabolism of material and useful work of various kinds. We can only refer to these important investigations which are of special physiological interest.

The action of muscular work on the gas exchange does not alone appear with hard work. From the researches of SPECK and others we learn that even very small, apparently quite unessential movements may increase the production of carbon dioxide to such an extent that by not observing these, as in numerous older experiments, very considerable errors may creep in. JOHANSSON[3] has also made experiments upon himself, and finds that on the production of as complete a muscular inactivity as possible the ordinary amount of carbon dioxide (31.2 grams per hour at rest in the ordinary sense) may be reduced nearly one third, or to an average of 22 grams per hour.

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[1] Zeitschr. f. Biologie, 2.

[2] See the works of Zuntz and Lehmann, Maly's Jahresber., 19; Katzenstein, Pflüger's Arch., 49; Loewy, *ibid.*; Zuntz, *ibid.*, 68, and especially the large work "Untersuch über den Stoffwechsel des Pferdes bei Ruhe und Arbeit," Zuntz and Hagemann, Berlin 1898, which also contains a bibliography. Zuntz and Slowtzoff, Pflüger's Arch., 95; Zuntz, *ibid.*

[3] Nord. Med. Arkiv. Festband, 1897; also Maly's Jahresber., 27; Speck, "Physiol. des menschl. Atmens," Leipzig, 1892.

The quantity of carbon dioxide eliminated during a working period is uniformly greater than the quantity of oxygen taken up at the same time, and hence a raising of the respiratory quotient was usually considered as caused by work. This rise does not seem to be based upon the character of chemical processes going on during work, as we have a series of experiments made by ZUNTZ and his collaborators, LEHMANN, KATZENSTEIN and HAGEMANN,[1] in which the respiratory quotient remained almost wholly unchanged in spite of work. According to LOEWY[2] the combustion processes in the animal body go on in the same way in work as in rest, and a raising of the respiratory quotient (irrespective of the transient change in the respiratory mechanism) takes place only with insufficient supply of oxygen to the muscles, as in continuous fatiguing work or excessive muscular activity for a brief period, also with local lack of oxygen caused by excessive work of certain groups of muscles. This varying condition of the respiratory quotient has been explained by KATZENSTEIN by the statement that during work two kinds of chemical processes act side by side. The one depends upon the work which is connected with the production of carbon dioxide also in the absence of free oxygen, while the other brings about the regeneration which takes place by the taking up of oxygen. When these two chief kinds of chemical processes make the same progress the respiratory quotient remains unchanged during work; if by hard work the decomposition is increased as compared with the regeneration, then a raising of the respiratory quotient takes place. If, on the contrary, moderate work is continued and performed in a way so that irregularities and occasional changes in the circulation and respiration are excluded or are without importance, then the respiratory quotient may correspondingly remain the same during work as in rest. Its extent is thereby in the first place determined by the nutritive material at its disposal (ZUNTZ and his pupils).

The theory of LOEWY and ZUNTZ, that the raising of the respiratory quotient during work is to be explained by an insufficient supply of oxygen, is opposed by LAULANIÉ.[3] He has observed the reverse, namely, a diminution in the respiratory quotient during continuous excessive work, and this is not reconcilable with the above statements. According to LAULANIÉ, who considers sugar as the source of muscular energy, the rise in the respiratory quotient is due to an increased combustion of sugar. The diminution of the same he explains by a re-formation of sugar from fat which takes place at the same time and is accompanied by an increased consumption of oxygen.

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[1] See footnote 2, page 759.

[2] Pflüger's Arch., 49.

[3] Arch. de Physiol. (5), 8, 572.

In *sleep* metabolism decreases as compared with that during waking, and the most essential reason for this is the muscular inactivity during sleep. The investigations of RUBNER upon a dog, and of JOHANSSON[1] upon human beings, teach us that if the muscular work is eliminated the metabolism during waking is not greater than in sleep.

The action of *light* also stands in close connection with the question of the action of muscular work. It seems positively proved that metabolism is increased under the influence of light. Most investigators, such as SPECK, LOEB, and EWALD,[2] consider that this increase is due to the movements caused by the light or an increased muscle tonus. FUBINI and BENIDICENTI[3] assume that the increase in metabolism due to light is independent of the movements. They base this assumption on experiments made on hibernating animals.

Mental activity does not seem to have any influence on metabolism according to the means at hand for studying this influence.

Action of the External Temperature. In cold-blooded animals the production of carbon dioxide increases and decreases with the rise and fall of the surrounding temperature. In warm-blooded animals this condition is different. By the investigations of LUDWIG and SANDERS-EZN, PFLÜGER and his pupils, and Duke CHARLES THEODORE of Bavaria and others[4] it has been demonstrated that in warm-blooded animals the change in the external temperature has different results according as the animal's own heat remains the same or changes. If the temperature of the animal sinks, the elimination of carbon dioxide decreases; if the temperature rises, the elimination of CO_2 increases. If, on the contrary, the temperature of the body remains unchanged, then the elimination of carbon dioxide increases with a lower and decreases with a higher external temperature. The statements on this subject are somewhat disputed and cases have been observed where in warm-blooded animals the metabolism rises on cooling and lowering the body temperature, while warming and raising the body temperature produces a diminution (KRARUP[5]).

The increase in metabolism produced by a lowering of the external temperature is explained, according to PFLÜGER and ZUNTZ, by the statement that the low temperature, by exciting a reflex action on the sensitive nerves of the skin, causes an increased metabolism in the muscles with an increased production of heat, affecting the temperature of the body, while with a higher external temperature the reverse takes place. The experiments made upon animals are somewhat uncertain for several reasons, but the determinations of the oxygen absorption, as well as the elimination of CO_2, made by SPECK, LOEWY, and JOHANSSON [1] in human beings, have shown that cold does not produce any essential increase in the metabolism of man. The irritation caused by cold may reflexly cause a forced respiration with an action on the gas exchange, and weak reflex muscular movements, such as shivering, trembling, etc., may cause an insignificant increase in the elimination of carbon dioxide; in complete muscularinactivity cold seems to cause no increased absorption of oxygen or increased metabolism. EYKMAN'S [2] experiments upon inhabitants of the tropics also show the same result, namely, that in human beings no appreciable heat regulation occurs.

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[1] Rubner, Ludwig-Festschr., 1887; Loewy, Berl. klin. Wochenschr., 1891, 434; Johansson, Skand, Arch. f. Physiol., 8.

[2] Speck, l. c.; Loeb, Pflüger's Arch, 42; Ewald, Journ. of Physiol., 13.

[3] Cited from Maly's Jahresber., 22, 395.

[4] The pertinent literature may be found cited by Voit in Hermann's Handbuch, 6, and also by Speck, l. c.

[5] J. C. Krarup, Den omgifvende temperaturs indflydeke, etc., Inaug.-Diss. Kjöbenhavn, 1902. See also Falloise, Maly's Jahresber., 31; Predteschensky, *ibid;* Rubner, Arch. f. Hygiene, 38.

A very interesting and important question is the action of high altitude upon the oxidation processes, the economy of temperature, the protein exchange and the general metabolism. The results of the laborious and important investigations on this subject may be found in the large work of N. ZUNTZ, A. LOEWY, F. MÜLLER and W. CASPARI.[3]

Metabolism is increased by the *ingestion of food*, and ZUNTZ has calculated that in man the consumption of oxygen is raised on an average 15 per cent above the amount during rest for about six hours after taking a moderately hearty meal. This increase in the metabolism is caused, according to the generally accepted view, probably only by the increased work of the digestive apparatus on the partaking of food. RJASANTSEFF has shown that the extent of nitrogen elimination is proportioned to the intensity of the digestive work. It also follows from the works of MAGNUS-LEVY, KORAEN and JOHANSSON [4] that the proteins and to a lesser extent the carbohydrates even by themselves produce a rise in metabolism which does not seem to be true for the fats.

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[1] Speck, l. c.; Loewy, Pflüger's Arch., **46**; Johansson, Skand. Arch, f. Physiol., **7**.

[2] Virchow's Arch., **133**, and Pflüger's Arch., **64**.

[3] "Hohenklima und Bergwanderungen in ihrer Wirkung auf den Menschen," Berlin, 1906.

[4] Zuntz and Levy, "Beitrag zur Kenntniss d. Verdaulichkeit, etc., des Brodes," Pflüger's Arch., **49**; Magnus-Levy, *ibid.*, **55**; Koraen, Skand. Arch. f. Physiol., **11**, Johansson and Koraen, *ibid.*, **13**.

VI. The Necessity of Food by Man under Various Conditions.

Various attempts have been made to determine the daily quantity of organic food needed by man. Certain investigators have calculated from the total consumption of food by a large number of similarly fed individuals — soldiers, sailors, laborers, etc. — the average quantity of foodstuffs required per head. Others have calculated the daily demand of food from the quantity of carbon and nitrogen in the excreta or calculated it from the exchange of force of the person experimented upon. Others, again, have calculated the quantity of nutritive material in a diet by which an equilibrium was maintained in the individual for one or several days between the consumption and the elimination of carbon and nitrogen. Lastly, still others have quantitatively determined during a period of several days the organic foodstuffs consumed daily by persons of various occupations who chose their own food, by which they were well nourished and rendered fully capable of work.

Among these methods a few are not quite free from objection, and others have not as yet been tried on a sufficiently large scale. Nevertheless the experiments collected thus far serve, partly because of their number and partly because the methods correct and control one another, as a good starting-point in determining the diet of various classes and similar questions.

If the quantity of foodstuffs taken daily be converted into calories produced during physiological combustion, we then obtain some idea of the sum of the chemical energy which under varying conditions is introduced into the body. It must not be forgotten that the food is never completely absorbed, and that undigested or unabsorbed residues are always expelled from the body with the fæces. The gross results of calories calculated from the food taken must therefore, according to Rubner, be diminished by at least 8 per cent. This figure is true at least when the human being partakes of a mixed diet of about 60 per cent of the proteins as animal and about 40 per cent of the proteins as vegetable foodstuffs. With more one-sided vegetable food, especially when this is rich in undigestible cellulose, a much larger quantity must be subtracted.

The following summary contains a few examples of the quantity of food which is consumed by individuals of various classes of people under different conditions. In the last column we also find the quantity of living force which corresponds to the quantity of food in question, calculated as calories, with the above-stated correction. The calories are therefore net results, while the figures for the nutritive bodies are gross results.

	Proteins.	Fat.	Carbohydrates.	Calories.	Authority.
Soldier during peace	119	40	529	2784	PLAYFAIR.[1]
" light service......	117	35	447	2424	HILDESHEIM.
" in field	146	46	504	2852	"
Laborer	130	40	550	2903	MOLESCHOTT.
Laborer at rest.........	137	72	352	2458	PETTENKOFER and VOIT.
Cabinetmaker (40 years).	131	68	494	2835	FORSTER.[2]
Young physician	127	89	362	2602	"
" "	134	102	292	2476	"
Laborer (36 years)......	133	95	422	2902	"
English smith	176	71	666	3780	PLAYFAIR.
" pugilist	288	88	93	2189	"
Bavarian wood-chopper .	135	208	876	5589	LIEBIG.
Laborer in Silesia.......	80	16	552	2518	MEINERT.[3]
Seamstress in London...	54	29	292	1688	PLAYFAIR.
Swedish laborer	134	79	485	3019	HULTGREN and LANDERGREN.[4]
Japanese student.......	83	14	622	2779	EIJKMAN.[5]
Japanese shopman......	55	6	394	1744	TAWARA.[5]

We have a very large number of complete investigations upon the diet of people of different vocations in America but they are too extensive to enter into, hence we must refer to the original publications of ATWATER.[6]

It is evident that persons of essentially different weight of body who live under unequal external conditions must need essentially different food. It is also to be expected (and this is confirmed by the table) that not only the absolute quantity of food consumed by various persons, but also the relative proportion of the various organic nutritive substances, shows considerable variation. Results for the daily need of human beings in general cannot be given. For certain classes, such as soldiers, laborers, etc., results may be given which are valuable for the calculation of the daily rations.

Based on extensive investigations and a very wide experience, VOIT has proposed the following average quantities for the daily diet of adults.

	Proteins.	Fat.	Carbohydrates.	Calories.
For men	118 grams	56 grams	500 grams	2810

But it should be remarked that these data relate to a man weighing 70 to 75 kilos and who was engaged daily for ten hours in not too fatiguing labor.

The quantity of food required by a woman engaged in moderate work is about four-fifths that of a laboring man, and we may consider the following as a daily diet with moderate work:

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[1] In regard to the older researches cited in this table we refer the reader to Voit in Hermann's Handbuch, **6**, 519.

[2] *Ibid.*, and Zeitschr. f. Biologie, **9**.

[3] Armee-und Volksernährung, Berlin, 1880.

[4] Untersuchung über die Ernährung schwedischer Arbeiter bei frei gewählter Kost Stockholm, 1891. Maly's Jahresber., **21**.

[5] Cited from Kellner and Mori in Zeitschr. f. Biologie, **25**.

[6] Report of the Storrs Agric. expt. Station, Conn. 1891–1895 and 1896 and U. S. Report of Agriculture, Bull. **53**, 1898.

	Proteins.	Fat.	Carbohydrates.	Calories.
For women........	94 grams	45 grams	400 grams	2240

The proportion of fat to carbohydrates is here as 1:8–9. Such a proportion occurs often in the food of the poorer classes which live chiefly upon the cheap and voluminous vegetable food, while this ratio in the food of wealthier persons is 1:3-4. It would be desirable if in the above rations the fat was increased at the expense of the carbohydrates, but unfortunately on account of the high price of fat such a modification cannot always be made.

In examining the above numbers for the daily rations it must not be forgotten that the figures for the various foodstuffs are gross results. They consequently represent the quantity of these which must be taken in, and not those which are really absorbed. The figures for the calories are, on the contrary, net results.

The various foods are, as is well known, not equally digested and absorbed, and in general the vegetable foods are less completely consumed than animal foods. This is especially true of the proteins. When, therefore, VOIT, as above stated, calculates the daily quantity of proteins needed by a laborer as 118 grams, he starts with the supposition that the diet is a mixed animal and vegetable one, and also that of the above 118 grams about 105 grams are absorbed. The results obtained by PFLÜGER and his pupils BOHLAND and BLEIBTREU [1] on the extent of the metabolism of proteins in man with an optional and sufficient diet correspond well with the above figures, when the unequal weight of body of the various persons experimented upon is sufficiently considered.

As a rule, the more exclusively a vegetable food is employed, the smaller is the quantity of proteins in the same. The strictly vegetable diet of certain people, as that of the Japanese and of the so-called vegetarians, is therefore a proof that, if the quantity of food be sufficient, a person may exist on considerably smaller quantities of proteins than VOIT suggests. It follows from the investigations of HIRSCHFELD, KUMAGAWA and KLEMPERER, SIVÉN, and others (see page 750) that a nearly complete or indeed a complete nitrogenous equilibrium may be attained by the sufficient administration of non-nitrogenous nutritive bodies with relatively very small quantities of proteins.

If we bear in mind that the food of people of different countries varies greatly, and that the individual also takes essentially different nourishment according to the external conditions of living and the influence of climate, it is not remarkable that a person accustomed to a mixed diet can exist for some time on a strictly vegetable diet deficient in proteins. No one doubts the ability of man to adapt himself to a heterogeneously composed diet when this is not too difficult of digestion and is sufficient in quantity; also we cannot deny that it is possible for a man to exist also for a long time with smaller amounts of protein than VOIT suggests, namely 118 grams. Thus O. NEUMANN[1] experimented on himself during 764 days in three series of experiments, and his diet consisted of 74.2 grams protein, 117 grams fat, and 213 grams carbohydrates = 2367 gross calories, with a weight of 70 kilos and with ordinary laboratory work. These figures cannot be compared with those obtained by VOIT's worker, weighing 70 kilos, whose work was harder than a tailor's and easier than a blacksmith's; for example, the work of a mason, carpenter, or cabinet-maker. The very extensive investigations recently performed by CHITTENDEN[2] on the estimation of the extent of protein necessary are of great interest. These investigations upon a total of twenty-six persons extended over a period of five to twenty months and consisted of careful investigations and observations upon the manner of living, food taken, nitrogen elimination, and the ability of performing work. The different individuals were divided into three groups. The first consisted of five professional men (four assistants and one professor). The second group was composed of thirteen soldiers (of the sanitary corp of the United States army) which besides their daily work were given gymnastic exercises for six months. The third group consisted of eight athletic students who were trained in different kinds of sport.

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[1] Bohland, Pflüger's Arch., 36; Bleibtreu, *ibid.*, 38.

In all the persons experimented upon the original nitrogen content of the food, which corresponded to VOIT's value or were somewhat higher, was gradually reduced more or less. The total calories supplied were not increased above the original value but rather diminished to a reasonable extent. The bodily as well as the mental ability was repeatedly tested. As it is not possible to enter into the details of the investigation the following will be sufficient to show the results. With a diet corresponding to VOIT's values the amount of urine nitrogen per day was 16 grams, corresponding to a total protein catabolism in the body of 100 grams or 1.43 grams per kilo. The corresponding results for the above three groups may be found in the following table where for comparison HAMMARSTEN includes also the figures for VOIT's diet.

	Urine Nitrogen.		Catabolized Protein.		Protein per Kilo.	
	Min.	Max.	Min.	Max.	Min.	Max.
Group 1	5.69	8.99	35.6	56.19	0.61	0.86
Group 2	7.03	8.39	43.9	52.44	0.74	0.87
Group 3	7.47	11.06	46.7	69.10	0.75	0.92
Voit's figures	16		100		1.43	

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[1] Arch. f. Hygiene, **45**.

[2] R. H. Chittenden, Physiological Economy in Nutrition, New York, 1904.

The chief results from these investigations are that on partaking of amounts of protein much smaller than VOIT's figures, without changing the original supply of calories and indeed diminishing the same, the persons experimented upon remained not only in nitrogenous equilibrium, but remained in perfect health and were not only able to perform the ordinary work but were indeed regularly able to perform much greater work.

From these investigations which extended over a long period and were carried on with special care in exactitude, it cannot be denied that man can exist for a long time with much smaller quantities of protein than VOIT's figures call for which is also derived from the experience of vegetarians and from people living nearly entirely upon vegetable food. On the other hand it must not be forgotten that VOIT's figures represent average results not theoretically necessary but which have been shown to be the actual diet developed from habit, custom, conditions of life and climate, with sufficient nourishment and free selection for centuries in Middle and North Europe. A rational change in this food requirement based upon scientific facts is just as difficult to determine as it is to carry out practically. Certain standard figures for the general needs of nutrition cannot be established because the conditions in various countries are different and must necessarily be so. The numerous compilations (of ATWATER and others [1]) on the diet of different families in America have given the figures 97–113 grams protein for a man, and the very careful investigations of HULTGREN and LANDERGREN have also shown that the laborer in Sweden with moderate work and an average body weight of 70.3 kilos, with optional diet, partakes 134 grams protein, 79 grams fat, and 522 grams carbohydrates. The quantity of protein is here greater than is necessary, according to VOIT. On the other hand LAPICQUE [2] found 67 grams protein for Abyssinians and 81 grams for Malaysians (per body weight of 70 kilos), materially lower figures.

If we compare the figures on page 764 with the average figures proposed by VOIT for the daily diet of a laborer, it would seem at the first glance as if the food consumed in certain cases was considerably in excess of the need, while in other cases, as, for instance, that of a seamstress in London, it was entirely insufficient. A positive conclusion cannot, therefore, be drawn if we do not know the weight of the body, as well as the labor performed by the person, and also the conditions of living. It is certainly true that the amount of nutriment required by the body is not directly proportional to the body weight, for a small body consumes relatively more substance than a larger one, and varying quantities of fat may also cause a difference; but a large body, which must maintain a greater quantity, consumes an absolutely greater quantity of substance than a small one, and in estimating the nutritive need one must also always consider the weight of the body. According to VOIT, the diet for a laborer with 70 kilos body weight requires 40 calories for each kilo. EKHOLM[1] calculates, basing it upon his experiments, that for a man weighing 70 kilos, busied with reading and writing, the net calories are 2450 and the gross calories 2700, or 35 and 38.5 calories per kilo. In the ordinary sense for a resting man the general food requirement is calculated in round numbers as 30 calories for every kilo. The minimum figure for metabolism during sleep and in as complete rest as possible has been found by SONDÉN, TIGERSTEDT and JOHANSSON[2] to be 24–25 calories.

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[1] Atwater, Report of the Storrs Agric. Expt. Station, Conn., 1891–1895 and 1896; also Nutrition investigations at the University of Tennessee, 1896 and 1897; U. S. Dept. of Agriculture, Bull. **53**, 1898. See also Atwater and Byrant, *ibid.*, Bull. **75**; Jaffa, *ibid.*, **83**; Grindley, Sammis, and others, *ibid.*, **91**.

[2] Hultgren and Landergren, l. c.; Lapicque, Arch. de Physiol. (5), **6**.

As several times stated above, the demands of the body for nourishment vary with different conditions of the body. Among these conditions two are especially important, namely, work and rest.

In a previous chapter, in which muscular labor was spoken of, it was seen that all foodstuffs have nearly the same power of serving as a source for muscular work, and that the muscles, it seems, select that foodstuff which is supplied to them in the greatest quantity. As a natural sequence it is to be expected that muscular activity requires indeed an increased supply of foodstuffs, but no essential change in the relation of the same, as compared to rest.

Still this does not seem to hold true in daily experience. It is a well-known fact that hard-working individuals — men and animals — require a greater quantity of proteins in the food than less active ones. This contradiction is, however, only apparent, and it depends, as VOIT has shown upon the fact that individuals used to violent work are more muscular. For this reason a person performing severe muscular labor requires food containing a larger proportion of proteins than an individual whose occupation demands less violent exertion. Another fact is that the diet rich in proteins is often concentrated and less bulky, and also that in many cases of training a diet containing as little fat as possible is selected.

If we compare the results for the needs of food in work and rest which are obtained under conditions which can be readily controlled, it is found that the above statements are confirmed in general. As example of this the following tables gives the rations of soldiers in peace and in the field and the average figures from the detailed data of various countries.[1]

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[1] Skand. Arch. f. Physiol., **11**.

[2] Sondén and Tigerstedt, Skand. Arch. f. Physiol., **6**; Johansson, *ibid.*, **7**; Tigerstedt, Nord. Med. Arkiv. Festband, 1897.

	A. Peace Ration.			B. War Ration.		
	Proteins.	Fat.	Carbohydrates.	Proteins.	Fat.	Carbohydrates.
Minimum	108	22	504	126	38	484
Maximum	165	97	731	197	95	688
Mean	130	40	551	146	59	557

The following figures for the daily ration are obtained from the above averages:

	Proteins.	Fat.	Carbohydrates.	Calories.
In peace	130	40	551	2900
In war	146	59	557	3250

If we calculate the fat in its equivalent quantity of starch, then the relation of the proteins to the non-nitrogenous foods is:

In peace	1:4.97
In war	1:4.79

The relation in both cases is nearly the same. Similar results are obtained when we start with Voit's figures for a soldier in manœuvre A (hard work) and B (strenuous work) in war.

	Proteids.	Fat.	Carbohydrates.	Calories.
A.....................	135	80	500	3013
B	145	100	500	3218

The relation here, when the fat is recalculated as starch, in both cases is the same, or equal to 1:5.

If we calculate that portion of the total calories supplied which falls to each group of the foodstuffs, it is found that 16–19 per cent comes from the protein in rest as well as with medium and strenuous work. For the fat and the carbohydrates the variations are greater; the chief quantity of calories comes from the carbohydrates. Of the total calories 16–30 per cent comes from the fat and 50–60 per cent from the carbohydrates.

The importance of the food-demand for working individuals is shown by the figures given on page 764 for a wood-chopper in Bavaria. A need of more than 4000 calories occurs only seldom, and with very hard work the demand may rise even to 7000 calories (Atwater and Bryant, Jaffa [2]).

As more work requires an increase in the absolute quantity of food, so the quantity of food must be diminished when little work is performed.

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[1] Germany, Austria, Switzerland, France, Italy, Russia, and the United States. It is not known by the author whether these figures have been changed in the last few years in the various countries, and hence whether they must be modified or not.

[2] See footnote 1, page 767.

The question as to how far this can be done is of importance in regard to the diet in prisons and poorhouses. We give below the following as example of such diets:

	Proteins.	Fat.	Carbohydrates.	Calories.	
Prisoner (not working)	87	22	305	1667	Schuster.[1]
Prisoner (not working)	85	30	300	1709	Voit,
Man in poorhouse	92	45	332	1985	Forster.[2]
Woman in poorhouse	80	49	266	1725	"

The figures given by Voit are, he says, the lowest reported for a non-working prisoner. He considers the following as the lowest diet for old non-working people:

	Proteins.	Fat.	Carbohydrates.	Calories.
Men	90	40	350	2200
Women	80	35	300	1733

In calculating the daily diet it is in most cases sufficient to ascertain how much of the various foodstuffs must be administered to the body in order to keep it in the proper condition to perform the work required of it. In other cases it may be a question of improving the nutritive condition of the body by properly selected food; and there also are cases in which it is desired to diminish the mass or weight of the body by an insufficient nutrition. This is especially the case in obesity, and all the dietaries proposed for this purpose are chiefly starvation cures which will be shown below from those selected, namely, Harvey, Ebstein and Oertel's cure.

The oldest and most generally known diet cure for corpulency is that of Harvey, which is ordinarily called the Banting method. The principle of this cure consists in increasing, as far as possible, the consumption of the accumulated fat of the body by as limited a supply of fat and carbohydrates as practicable and a simultaneously increased supply of proteins. A second, called Ebstein's cure, based on the assumption (not correct) that the fat of the food is not accumulated in a body rich in fat, but is completely burnt. In this cure large quantities of fat are therefore allowed in the food, while the quantity of carbohydrates is diminished very materially. The third cure, called Oertel's[3] cure, is based on the correct view that a certain quantity of carbohydrates has no greater influence in the accumulation of fat than the isodynamic quantities of fat. In this cure, therefore, carbohydrates as well as fat are allowed, provided the total quantity of the same is not so great as to hinder the decrease in the fatty condition. A greatly diminished supply of water is also one of the features of OERTEL's cure, especially in certain cases. The average quantity of the various nutritive substances supplied to the body in these three cures is as follows, and we give also for comparison in the same table VOIT's diet necessary for a laborer.

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[1] See Voit, Untersuchung der Kost. München, 1877, 142. See also Hirschfeld Maly's Jahresber., 30.

[2] See Voit, Intersuchung, der Kost, page 186.

[3] Banting, Letter on Corpulence. London, 1864. Ebstein, Die Fettleibigkeit und ihre Behandlung. 1882. Oertel, Handbuch der allg. Therapie der Kreislaufstörungen. 1884.

	Proteins.	Fat.	Carbohydrates.	Calories (gross).
HARVEY-BANTING'S cure	171	8	75	1083
EBSTEIN'S cure	102	85	47	1396
OERTEL'S "	156	22	72	1140
" " (Max)	170	44	114	1573
Laborer, according to Voit	118	56	500	3055

If the fat in all cases is recalculated in starch, then the proportion of the proteins to the carbohydrates is;

HARVEY-BANTING'S cure	100 : 54
EBSTEIN'S cure	100 : 240
OERTEL'S "	100 : 80
" " (Max)	100 : 129
Laborer	100 : 530

In all these cures for corpulence the quantity of non-nitrogenous bodies is diminished as compared with the proteins; but also the total quantity of food, as is shown by the number of calories, is considerably diminished.

HARVEY-BANTING'S cure differs from the others in a relatively very much greater quantity of proteins, while the total number of calories in it is the smallest. On this account this cure acts very quickly; but it is therefore also more dangerous and more difficult to accomplish. In this regard EBSTEIN'S and OERTEL'S cures (especially OERTEL'S), having a greater variation in the selection of food, are better. As the adipose tissue has a protein-sparing action, we have to consider in using these cures, especially BANTING'S, that the destruction of proteins in the body is not increased in the adipose tissue, and one must therefore carefully watch the elimination of nitrogen by the urine. All diet cures for obesity are moreover, as above stated, starvation cures; and if the daily quantity of food required by an adult man, represented as calories, is in round numbers 2500 calories (according to the average figures found by FORSTER in the case of a physician), then one immediately sees what a considerable part of its own mass the body must daily give up in the above cures. This reminds us of the great care necessary in employing them; each special case should be conducted with regard to the individuality, the weight of the body, the elimination of nitrogen in the urine, etc., etc., and always under strong control, and only by a physician, never by a layman. A more detailed discussion of the many conditions which must be considered in these cases does not enter the plan and scope of this work.

TABLE I.—FOODS.[1]

I. Animal Foodstuffs.	1000 Parts contain						Relationship of 1:2:3.		
	1 Proteids and Extractives.	2 Fat.	3 Carbohydrates.	4 Ash.	5 Water.	6 Waste.	1	:2	:3
a. MEAT WITHOUT BONES.									
Fat beef [2]	183	166		11	640		100	90	0
Beef (average fat [1])	196	98		18	688		100	50	0
Beef [2]	190	120		18	672		100	63	0
Corned beef (average fat)	218	115		117	550		100	53	0
Veal	190	80		13	717		100	42	0
Horse, salted and smoked	318	65		125	492		100	20	0
Smoked ham	255	365		100	280		100	143	0
Pork, salted and smoked [3]	100	660		40	130		100	660	0
Meat from hare	233	11		12	744		100	5	0
" " chicken	195	93		11	701		100	48	0
" " partridge	253	14		14	719		100	6	0
" " wild duck	246	31		12	711		100	13	0
b. MEAT WITH BONES.									
Fat beef [2]	156	141		9	544	150	100	90	0
Beef (average fat [1])	167	83		15	585	150	100	49	0
Beef, slightly corned	175	93		85	480	167	100	53	0
Beef, thoroughly corned	190	100		100	430	180	100	53	0
Mutton, very fat	135	332		8	437	88	100	246	0
" average fat	160	160		10	520	150	100	100	0
Pork, fresh, fat	100	460		5	365	70	100	460	0
" corned, fat	120	540		60	200	80	100	450	0
Smoked ham	200	300		70	340	90	100	150	0
c. FISHES.									
River eel, fresh, entire	89	220		6	352	333	100	246	0
Salmon, " "	121	67		10	469	333	100	56	0
Anchovy, " "	128	39		11	489	333	100	31	0
Flounder, " "	145	14		11	580	250	100	9	0
River perch, fresh, entire	100	2		8	440	450	100	2	0
Torsk, " "	86	1		8	455	450	100	1	0
Pike, " "	82	1		6	461	450	100	1	0
Herring, salted, entire	140	140		100	280	340	100	100	0
Anchovy "	116	43		107	334	400	100	37	0
Salmon (side), salted	200	108		132	460	100	100	54	0
Kabeljau (salted haddock)	246	4		178	472	100	100	1	0
Codfish (dried ling)	532	5		106	257	100	100	1	0
" (dried torsk)	665	10		59	116	150	100	1	0
Fish-meal from variety of GADUS	736	7		87	170		100	1	0

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[1] The results in the following tables are chiefly compiled from the summary of ALMÉN and of KÖNIG. We here designate as "waste" that part of the foods which is lost in the preparation or that which is not used by the body; for instance, bones, skin, egg-shells, and the cellulose vegetable foods.

[2] Meat such as is ordinarily sold in the markets in Sweden.

[3] Pork, chiefly from the breast and belly, such as occurs in the rations of Swedish soldiers.

TABLE I.—FOODS—(*Continued*).

	1000 Parts contain						Relationship of 1:2:3		
	1	2	3	4	5	6	1	2	3
1. Animal Foodstuffs.	Proteids and Extractives.	Fat.	Carbohydrates.	Ash.	Water.	Waste.			
d. INNER ORGANS (FRESH).									
Brain	116	103		11	770		100	89	0
Beef-liver	196	56	11	17	720		100	28	0
Beef-heart	184	92		10	714		100	50	0
Heart and lungs of mutton	163	106		10	721		100	65	0
Veal-kidney	221	38		13	728		100	17	0
Ox tongue (fresh)	150	170		10	670		100	113	0
Blood from various animals (average results)	182	2		9	807		100	1	0
e. OTHER ANIMAL FOODS.									
Variety of pork-sausage (Mettwurst)	190	150		50	610		100	79	0
Same for frying	220	160		55	565		100	73	0
Butter	7	850	7	15	119		100	12100	100
Lard	3	990			7		100	33000	0
Meat extract	304			175	217				
Cow's milk (full)	35	35	50	7	873		100	100	143
" " (skimmed)	35	7	50	7	901		100	20	143
Buttermilk	41	9	38	7	905		100	22	93
Cream	37	257	35	6	665		100	695	95
Cheese (fat)	230	270	40	60	400		100	117	17
" (poor)	334	66	50	50	500		100	19	15
Whey cheese (poor)	89	70	456	56	329		100	79	512
Hen's egg, entire	106	93	4	8	654	135	100	88	4
" " without shell	122	107	5	10	756		100	88	4
Yolk of egg	160	307		13	520		100	192	0
White of egg	103	7	7	8	875		100	7	7
2. Vegetable Foodstuffs.									
Wheat (grains)	123	17	676	18	140	26	100	14	549
Wheat-flour (fine)	110	10	740	8	120	12	100	11	654
" " (very fine)	92	11	768	3	120	6	100	12	835
Wheat-bran	150	39	439	50	130	192	100	26	292
Wheat-bread (fresh)	88	10	550	17	330	5	100	11	625
Macaroni	90	3	768	8	131		100	3	853
Rye (grains)	115	17	688	18	140	22	100	15	600
Rye-flour	115	15	720	20	110	20	100	13	626
Rye-bread (dry)	114	20	725	15	110	16	100	18	634
" " (fresh, coarse)	77	10	480	16	400	17	100	14	623
" " (fresh, fine)	80	14	514	11	370	11	100	18	634
Barley (grains)	111	21	654	26	140	48	100	19	589
Scotch barley	110	10	720	7	146	7	100	9	654
Oat (grains)	117	60	563	30	130	100	100	51	481
" (peeled)	140	60	660	20	100	20	100	43	471
Corn	101	58	656	17	140	28	100	57	662
Rice (peeled for boiling)	70	7	770	2	146	5	100	10	1100
French beans	232	21	537	36	137	37	100	9	231
Peas (yellow or green, dry)	220	15	530	25	150	60	100	7	240
Flour from peas	270	15	520	25	125	45	100	6	192

TABLE I.—FOODS—(*Continued*).

2. Vegetable Foodstuffs.	1000 Parts contain						Relationship of		
	1 Proteids and Extractives.	2 Fat.	3 Carbohydrates.	4 Ash.	5 Water.	6 Waste.	1	2	3
Potatoes	20	2	200	10	760	8	100	10	1030
Turnips	14	2	74	7	893	10	100	14	529
Carrot (yellow)	10	2	90	10	873	15	100	20	900
Cauliflower	25	4	50	8	904	9	100	16	200
Cabbage	19	2	49	12	900	18	100	11	258
Beans	27	1	66	6	888	12	100	4	244
Spinach	31	5	33	19	908	8	100	16	106
Lettuce	14	3	22	10	944	7	100	21	157
Cucumbers	10	1	23	4	956	6	100	10	230
Radishes	12	1	38	7	934	8	100	8	317
Edible mushrooms (average)	32	4	60	9	877	18	100	12	188
Same dried in the air (average)	219	25	412	61	160	123	100	12	188
Apples and pears	4		130	3	832	31	100		3250
Various berries (average)	5		90	6	849	50	100		1800
Almonds	242	537	72	29	54	66	100	222	30
Cocoa	140	480	180	50	55	95	100	343	129

TABLE II.—MALT LIQUORS.

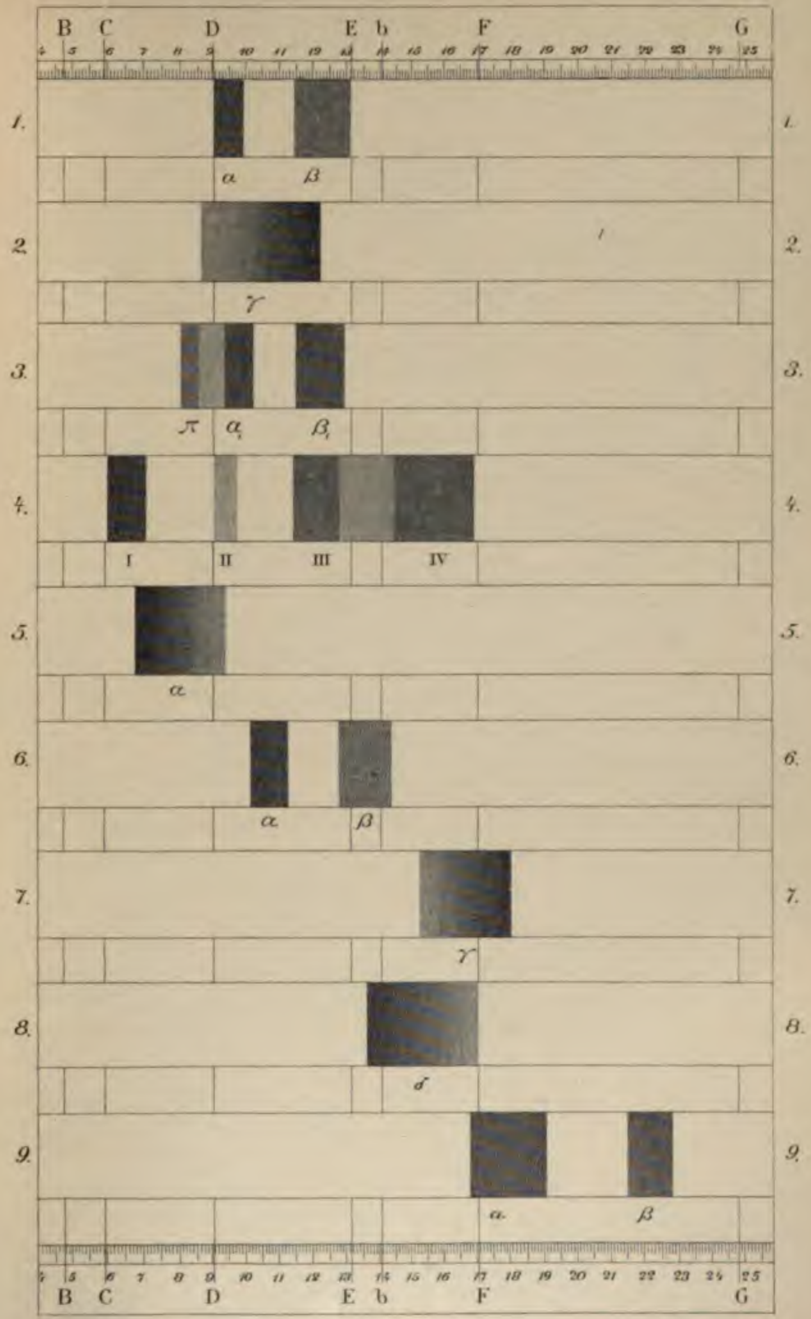
1000 Parts by Weight contain	Water.	Carbon Dioxide.	Alcohol.	Extract.	Proteids.	Sugar.	Dextrin.	Acids.	Glycerine.	Ash.
Porter	871	2	54	76	7	13		3.0	—	4
Beer (Swedish)	887		28	—	15	65		—	—	5
" (Swedish export)	885		32	—	7	73		—	—	3
Draught-beer	911	2	35	55	8	10	31	2.0	2	2
Lager-beer	903	2	40	58	4	7	47	1.5	2	2
Bock-beer	881	2	47	72	6	13	—	1.7	—	3
Weiss-beer	916	3	25	59	5	—	—	4.0	—	2
Swedish "Svagdricka"	945	—	22	—	7	23		—	—	3

TABLE III.—WINES AND OTHER ALCOHOLIC LIQUORS.

1000 Parts by Weight contain	Water.	Alcohol, Vol. Per Cent.	Extract.	Sugar.	Acid and Potassium Bitartrate.	Glycerine.	Ash.	Carbon Di-oxide, Vol Per Cent.
Bordeaux wine	883	94	23	6	5.9	—	2.0	
White wine (Rheingau)	863	115	23	4	5.0	—	2.0	
Champagne	776	90	134	115	6.0	1.0	1.0	60–70
Rhine wine (sparkling)	801	94	105	87	6.0	1.0	2.0	
Tokay	808	120	72	51	7.0	9.0	3.0	
Sherry	795	170	35	15	5.0	6.0	5.0	
Port wine	774	164	62	40	4.0	2.0	3.0	
Madeira	791	156	53	33	5.0	3.0	3.0	
Marsala	790	164	46	35	5.0	4.0	4.0	
Swedish punch	479	263	—	332	—	—	—	
Brandy	—	460	—	—	—	—	—	
French cognac	—	550	—	—	—	—	—	
Liqueurs	—	442–590	—	260–475	—	—	—	

SPECTRUM PLATE.

1. Absorption spectrum of a solution of *oxyhæmoglobin.*
2. Absorption spectrum of a solution of *hæmoglobin,* obtained by the action of an ammoniacal ferro-tartrate solution on an oxyhæmoglobin solution.
3. Absorption spectrum of a faintly alkaline solution of *methæmoglobin.*
4. Absorption spectrum of a solution of *hæmatin* in ether containing oxalic acid.
5. Absorption spectrum of an alkaline solution of *hæmatin.*
6. Absorption spectrum of an alkaline solution of *hæmochromogen,* obtained by the action of an ammoniacal ferro-tartrate solution on an alkaline-hæmatin solution.
7 Absorption spectrum of an acid solution of *urobilin.*
8. Absorption spectrum of an alkaline solution of *urobilin* after the addition of a zinc chloride solution.
9 Absorption spectrum of a solution of *lutein* (ethereal extract of the egg-yolk).


B C D E b F G
4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
1. α β
2. γ
3. π α₁ β₁
4. I II III IV
5. α
6. α β
7. γ
8. δ
9. α β

GENERAL INDEX.

Abietinic acid, 336
Abiuret products, 54, 394, 417
Absorption, 412–427
  , action of putrefactive processes in the intestine on, 405–407
Absorption ratio, 218
  of the blood pigments, 218
Acceptor, 6
Acetanilid, behavior in animal body, 633
Acethæmin, 212
Acetic acid in intestinal contents, 400
  in gastric contents, 373, 376
  , passage of, into urine, 608, 629
Aceto-acetic acid, 625, 671
  in urine, 625, 668
Acetone, 32, 669,
  in urine, 668
Acetonuria, 668, 669
Acetophenone, behavior in body, 367
Acetylene, compound with hæmoglobin, 267
Acetyldichitosamine, 686
Acetyl equivalent, 137
Acetyl acid equivalent, 137
Acetylamino benzoic acid, 636
Acetylparaminophenol, 633
Achilles tendon, composition of, 429
Acholia, pigmentary, 329
Achromatin, 149
Achroo-dextrin, 128
Acid albuminates, 36
  , properties, 48, 49
  , formation in peptic digestion, 55, 359
  , absorption of, 413
Acid amines, behavior in the animal body, 630
Acid equivalent, 137
Acid fermentation of urine, 677
Acid hæmoglobin, 205
Acid rigor, 466
Acids, organic, behavior in the animal body, 624, 629–631
Acidity of urine, 544, 545
  of the gastric contents, 374
  of the muscles, 448, 466, 467, 469,
Acrite, 114
Acrolein, 132
Acrolein test, 132, 136
Acroses, 114
Acrylic acid diureid. See Uric acid.
Actiniochrom, 691
Adamkiewicz-Hopkins reaction, 42, 103
Adelomorphic cells, 349, 364,
Adenase, 16, 271, 273
Adenine, 157, 162, 271, 571, 578
  , properties, reaction, 162
  , in urine, 162, 578
Adhesion, importance in blood coagulation, 227
Adipocere, 442
Adrenalin, 278, 279
  , relation to glycosuria, 298
Adrenalin-like bodies, 278
Ægagropila, 411
Ærotonometric method, 709
Age, influence on metabolism, 755-758
Agglutination, 195
Agglutines, 26, 195
Alanine, 26, 33, 54, 84, 305, 462
Alanylalanine, 396
Alanylglycine, 55, 396
Alanylleucine, 396
Albumin, 36, 45
  , detection of, in urine, 640, 643
  , quantitative estimation of, 645
  . See also Proteids.
Albumins, 36
  , general properties, 39, 45, 60
  . See also the various albumins.
Albuminates, 36
  , properties, 49, 48
  , ferruginous albuminate in the spleen, 272
Albuminoids, 37, 72–82, 430, 433, 492
Albumoids. See Albuminoids.
Albuminose, in spermatozoa, 498
Albuminous bodies. See Proteids.
Albuminuria, 640
  , alimentary, 413
Albumoses. See Proteoses.
Alcapton and alcaptonuria, 591, 597, 599
Alcohol. See Ethyl alcohol.
Alcoholase, 21
Alcoholic fermentation. See Ethyl alcohol.
Aldehydases of the liver, 18
Aldehydes, 105
  , behavior in the animal body, 631, 636
Aleuron grains, 503
Alexines, 186
Aldoses, 105
Alimentary glycosuria, 299, 420

Alizarin, in the urine, 639
    , administration of, 438
Alkali albuminates, 36, 48, 49
    , absorption of, 413
Alkali albumose, 49
Alkali carbonates, physiological importance for gaseous exchange, 698–702
    , action on secretion of gastric juice, 350
    , action on secretion of pancreatic juice, 384
    . See various tissues and fluids.
Alkalies, relation to gaseous exchange, 223, 224
    , diffusible and non-diffusible in blood, 224
    , division of, in blood corpuscles and plasma, 224, 238
    . See also the various fluids and tissues.
Alkali phosphates in urine, 619–622, 720
    , occurrence. See the various fluids and organs.
Alkali proteose, 49
Alkali urates, 542, 575
    in calculi, 680
    in sediments, 542, 575, 677, 678
Alkali earths, elimination by the intestine, 619, 626
    in urine, 619, 620, 626
    in bones, 436
    insufficient supply of, 438, 439, 727, 738
Alkaline fermentation of urine, 677
Alkalinity, determination of, in blood, 191
Alkaloids, action on muscles, 466
    , passage of, into urine, 639
    , retention by the liver, 280
Alkyl sulphide of the skunk, 692
Allantoin, properties and occurrence, 568, 573, 583, 584
    in transudates, 259, 513
    , formation from uric acid, 568, 574
Alloxan, 568
Alloxuric bases, 156, 578
Alloxuric bodies, 156
Alloxyproteic acid, 611, 613
Almén-Böttger-Nylander's sugar test, 116 655
Ambergris, 412
Ambrain, 412
Amide nitrogen, 26, 27
Amino acids, 88–103
    , relation to formation of uric acid, 572
    , relation to formation of urea, 550–553, 630
    , relation to carbohydrate metabolism, 305
Amino acids, formation from protein substances, 30, 33, 54, 380, 394, 401, 416
    , deamidation of, 305, 462, 550, 571
    , passage of, in the urine, 614, 675
    , conjugation of, 34
Amino-acetic acid. See Glycocoll.
Amino-benzoic acids, behavior in the animal body, 636
Amino-caproic acid. See Leucine.
Amino-cerebrinic acid chloride, 486
Amino-cerebrinic acid glucoside, 486
Amino-cinnamic acid, 633, 635
Amino-glutaric acid. See Glutamic acid.
Amino-ethyl sulphonic acid. See Taurine.
Amino-phenyl-acetic acid, behavior in animal body, 634
Amino-phenyl-propionic acid, 30, 585
Amino-phenyl-propionic acid, behavior in the animal body, 585, 633
Amino-propionic acid, 84. See also Alanine.
Amino-pyrotartaric acid. See Glutamic acid.
Amino sugar, 107. See also Glucosamine.
Amino-succinic acid. See Aspartic acid.
Amino-thiolactic acid. See Cysteine.
Amino-valerianic acid, 84
Amidulin, 126
Ammonia, formation in autolysis, 380
    , formation in protein putrefaction, 401
    , formation from protein substances, 26, 29, 380, 394, 401 544
    , formation in tryptic disection, 394
    , occurrence in blood, 241, 551
    , occurrence in urine, 544, 549, 623
    , elimination after administration of mineral acids, 544, 624, 625
    , elimination in disease, 549
    , elimination in diseases of the liver, 554
    , after extirpation or atrophy of the liver, 554
Ammonia, estimation of, in urine, 623, 624
Ammonium salts, relation to formation of glycogen, 291
    , relation to formation of urea, 572
    , relation to formation of uric acid, 551
Ammonium-magnesium phosphate in urinary calculi, 678, 682
Ammonium-magnesium phosphate in intestinal calculi, 411
Ammonium-magnesium phosphate in urinary sediment, 679

Ammonium sulphate, method of separating proteoses, 38, 51, 60
, method of separating carbohydrates, 289
Ammonium urate in urinary sediments, 681
in urinary calculi, 677, 678
Amniotic fluid, 512
Amphicreatine, 457
Amphopeptone, 51, 57
Amygdalin, 17
Amylose, 126
Amylodextrin, 126, 128
Amyloid, 36, 69, 431
, vegetable, 129
Amyloid degeneration, bile in, 329
, chondroitin-sulphuric acid in liver in, 431
Amylolytic enzymes, 16, 386, 387. See also various tissues and secretions.
Amylopectin, 126
Amylopsin, 387, 388
Amylum. See Starch.
Anæmia. pernicious, 246
Anærobic metabolism, 21, 461
Aniline, behavior in the animal body, 633
Anisotropous substance, 447
Antedonin, 691
Antialbumate, 359
Antialbumid, 56, 359
Antialbumose, 51
Antienzymes, 210. See also various enzymes.
Antifebrine, relation to elimination of urobilin, 604
Antimony, passage of, into milk, 539
, action on the elimination of nitrogen, 548
Antipeptone, 51, 54, 57
Antipyrine, relation to formation of glycogen, 291
, action on the urine, 604, 638, 639
Antitoxines, 25
Antoxyproteic acid, 611, 612
Anuria, in cholera, 694
Aorta elastin, 75, 76
Apatite in bone-earths, 436
Aqueous humor, 264
Arabinose, 108, 112
, relation to formation of glycogen, 290
Arabinosimine, 108
Arabite, 106
Arachidic acid, 131, 518
Arachoidal fluid, 257
Arbacin, 62
Arbutin, relation to formation of glycogen, 291, 592
, behavior in the animal body, 592
Arginase, 16, 21, 36, 271, 284, 550
Arginine, 16, 59, 63, 96, 550
Argon in blood, 697
Arnold's aceto-acetic acid reaction, 672
Aromatic compounds, behavior in animal body, 632–639
Aromatic substances in the urine, 586
Arsenic, in the animal body, 187, 239, 685, 695
action on the elimination of nitrogen, 548
Arsenious acid, action on peptic digestion, 359
Arseniuretted hydrogen, poisoning with, 331–333
Arterin, 196
Ascitic fluids, 259, 262, 263
Asparagine, 88
, relation to formation of glycogen, 291
, nutritive value, 747
Asparaginic acid. See Aspartic acid.
Aspartic acid, 87
, relation to formation of uric acid, 572
, relation to formation of urea, 550
, formation from protein, 33, 54, 88
, behavior in the organism, 550, 572, 630
Asparagus, odoriferous bodies of, in the urine, 639
Assimilation limit, 259, 260
Ass's milk, 529
Atmidalbumin, 52
Atmidalbumose, 52
Atmidkeratin, 75
Atmidkeratose, 75
Atropine, action of, elimination of uric acid, 569
, on the secretion of saliva, 347
Auto-digestion, 372. See Autolysis.
Auto-intoxication, 25
Autolysis, 22, 23
, substances retarding coagulation produced in, 234
. See also the various organs and tissues.
Auto-oxidation, 3–8

Bacterial proteins, 28
Bacterium ureæ, 677
Banting cure, 770
Beer-vinegar bacteria, enzyme of, 11
Beeswax, 138
Béla's acetone reaction, 671
Bence-Jones proteid, 645
Benzaldehyde, oxidation of, 5, 6
, substituted aldehydes, behavior in the animal body, 636
Benzoic acid, formation from protein substances, 31, 32, 585
, passage of, into sweat, 695

Benzoic acid, behavior in the organism, 3, 585, 635
, occurrence in the urine, 586
, substituted benzoic acids, action in body, 635
Benzene, 31, 76
, behavior in the animal body, 632, 633
Benzoyl-amino-acetic acid. See Hippuric acid.
Benzoyl-cystine, 93
Benzoar-stones, 411
Bial's reagent, 667
Bifurcated air, 706
Bile, 307-334
, analysis of, 326, 327
, antiseptic action, 405-407
, enzymes of, 325
, in disease, 329
, influence on protein digestion, 358, 393, 394, 398, 399
, on the secretion of bile, 309
, on the absorption of fat, 398, 405, 406, 422-425
, on tryptic digestion, 393, 394, 399
, molecular concentration of, 326
, passage of foreign bodies, 329
, occurrence of, in urine, 426, 652
, occurrence of, in gastric contents, 373, 398, 399
in meconium, 410
, decomposition in the intestine, 403
, chemical formation of, 329-333
Bile-concretions, 333, 334
Bile-pigments, 320-325
, origin and formation, 329-333
, reactions, 321, 322, 652, 653
, passage of, into urine, 652
Biliary fistulæ, 307, 406
, influence of, on intestinal putrefaction, 406
, influence on the food requirement, 406, 407
Bile-salts, 310
Bile-acids, 311, 319
in urine 426, 652
, detection of, 319, 652
, absorption of, 426
, origin of, 330, 331
, Pettenkofer's test for, 311
Bile-mucus, 310, 329
Bilianic acid, 315
Bilicyanin, 319, 322, 324
Bilifulvin, 320
Bilifuscin, 319, 324
Bilihumin, 319, 324
Biliphæin, 320
Biliprasin, 319, 324
Bilipurpurin, 324
Bilirubin, 319, 320
, relationship to blood-pigments, 210, 215, 319, 331, 333
, relationship to hæmatoidin, 216, 320, 321
Bilirubin, putrefaction of, 400
, occurrence of, 319
Biliverdin, 323
in fæces, 409
Biogen molecule, 4
Biogens, 4
Biological protein reaction, 186, 413
Bismuth, passage of, into milk, 539
Birotation, 88, 109
Bitch's milk, 529, 535
Biuret, 34, 555
Biuret base, 35; cleavage of, 395
Biuret reaction, 43, 44, 50, 555
Bladder. See Urinary calculi.
Bleeding, 248, 298, 697
Blister fluid, 265
Blonds, milk of, 534
Blood, 170-249
, general behavior, 170, 222, 225
, analyses, quantitative, 235-241
, analyses, physico-chemical, 191, 236
, arterial and venous, 196, 197, 241, 697
, defibrinated, 172
, asphyxiation, 197, 697
, quantity of, in the body, 248
, detection, chemico-legal, 216
, distribution of, in the organs, 249
, behavior in starvation, 244
, composition under various conditions, 241-247
in gastic contents, 373
in urine, 648, 650
Blood-casts, 648
Blood-clot, 172, 225
Blood coagulation, 170, 171, 176-178, 225-235
Blood-corpuscles, white, 220, 221, 226, 227
, number of, 220, 225, 226, 246, 247
, relation to coagulation, 220
, red, 192, 193
, number of, 192, 244, 246
, relation to high altitudes, 245
, passage of, into urine, 648
, permeability of, 195
, composition of, 218, 219
Blood gases, 696, 702
Blood-pigments, 195-221
in bile, 329
in urine, 648, 649
, estimation, 217, 218
, regeneration, 216
Blood-plasma, 172-183
, composition of, 187, 188, 238-240
Blood-plates, 220-222, 227, 228
, relation to coagulation of blood, 227, 231-233
Blood-serum, 172, 183-192

Blood-serum, composition of, 188–192
Blood-spots, 217
Blood transfusion, 245, 247, 248
Blueberries, coloring matter of, in urine, 639
Blue stentorin, 691
Boar spermatozoa, 497
Boas' reaction for HCl, 374
  for lactic acid, 374
Bones and bone tissues, 434, 440
  in starvation, 620
Bone-earths, 436, 437
Bone marrow, 172, 437
Bonellin, 691
Borneol, 609, 638
Böttcher's spermine crystals, 496
Böttger-Almén-Nylander's sugar test, 94, 116, 655
Bowman's disks, 488
Brain, 479–489
Bromadenine, 159
Bromanil, 32
Bromhypoxanthine, 159
Bromides, behavior to secretion of gastric juice, 364
Bromine, passage of, into saliva, 346, 347
Bromoform, from protein, 32
  , behavior in the animal body, 631
Bromtoluene, behavior in the animal body, 636
Brunettes, milk of, 534
Brunner's glands, 377
Buccal mucus, 341
Buffy coat, 225
Bufidin, 692
Bufotalin, 692
Bufotenin, 692
Bufotin, 692
Bull, spermatozoa, 497
Burbot, spermatozoa, 62
Bursæ mucosæ, contents of, 266
Butalanine, 81, 382
Butter-fat, 517, 518
  , absorption of, 424
Butterfly, pigment of wings, 569, 690
Buttermilk, 528
Butyl alcohol, behavior in the animal body, 632
Butyric acid, in urine, 607
  in gastric contents, 376
  in milk fat, 517, 518
Butyric-acid fermentation, 5, 110, 516
  in intestine, 397, 403
Butylmercaptan, 692
Butyrinase, in blood, 185
Byssus, 37, 81

Cadaver alkaloids, 24
Cadaverine, 24, 98, 457
  in intestine, 676
  in urine, 615, 676
Cæcum, solution of cellulose in the, 397, 398
Caffeine, 157
  , action on the muscles, 466
  , behavior in the animal body, 579
Calcium, lack of, in food, 438, 439, 737
  , occurrence, 625. See also various tissues and fluids.
Calcium carbonate in urine, 679
  in urinary calculi, 682
  in urinary sediments, 678
  in bones, 436, 439
  in tartar, 348
  in otoliths, 494
Calcium casein, 441
Calcium oxalate in urine, 582
  in urinary sediments, 678
  in urinary calculi, 681, 682
Calcium phosphate, relation to the coagulation of fibrinogen, 229
  , relation to the coagulation of casein, 520
  , occurrence in the intestinal concretions, 410, 411
  in the urine, 543, 619, 620, 625
  in urinary sediments, 679, 680
  in urinary calculi, 681 682
Calcium salts, elimination, 619, 626
  , importance to coagulation of the blood, 171, 177, 229
  , importance to coagulation of milk, 520
  . See various calcium salts.
Calcium sulphate, in urinary sediments, 679
  , ion action, 168
Calculi, salivary, 348
  , intestinal, 410–412
  , urinary, 680–683
Calories of foodstuffs, 723–727
  of different rations, 763–771
Campho-glucuronic acid, 122, 610, 638
Camphor, behavior in the animal body, 610, 638
Cane-sugar. See Saccharose.
Capranica's reaction for guanine, 161
Capric acid, 131, 518, 530
Caproic acid, 131, 518, 530
Caprylic acid, 131, 518
Caramel, 115, 124
Carbamino acetic acid, 701
Carbamic acid, 563
  in blood, 186, 552
  in urine, 552, 553, 563
  poisonous action, 552
Carbamic-acid ethylester, 563
Carbazol, behavior in body, 634
Carboglobulinic acid, 701
Carbohæmoglobins, 207

Carbohydrates, 104-130
, importance in fat formation, 444, 445, 753
, importance in glycogen formation, 290, 292, 293
, importance for muscular activity, 459, 468, 469, 473, 474
, action on protein metabolism, 738, 747, 748, 749, 751
, action on intestinal putrefaction, 415, 406, 589
, absorption of, 419-421
, inadequate supply of, 739
. See also the various carbohydrates.
Carbolic acid, action on peptic digestion, 359
. See also Phenol.
Carbolic urine, 591
Carbon, relation to nitrogen in the urine, 628, 720
, calorific value, 723
Carbon dioxide, assimilation, 1
in the blood, 696-701, 708-710, 712
in the blood in diabetes, 701, 702,
in the blood in poisoning with mineral acids, 701
in the intestine, 401, 403
in the lymph, 251, 702
in the stomach, 372
in the muscles during rest and activity, 468, 472
in the muscles in rigor mortis, 466
in the secretions, 702, 703
in transudations, 703
action on the secretion of gastric juice, 353
elimination, dependence of external temperature upon, 761
elimination in rest and activity, 468, 472, 759, 760
elimination by the skin, 695
elimination in the incubations of the egg, 510
elimination in various ages, 756-758
Carbon-dioxide hæmoglobin, 207, 699
Carbon-monoxide poisoning, 205, 299, 460, 548
action on the formation of lactic acid, 460
action on the elimination of nitrogen, 548
action on the elimination of sugar, 299, 460
Carbon monoxide hæmochromogen, 209
Carbon-monoxide methæmoglobin, 207
Carbon-monoxide blood test, Hoppe-Seyler's, 206
Carminic acid, 690
Carnic acid, 457
Carniferrine 457
Carnine, 158, 456, 457
, in urine, 578
Carnitine, 457
Carnomuscarine, 457
Carnosine, 454
Carp, sperma of, 63, 504
, eggs of, 71
Cartilage, 69, 431-434
, quantity of ash, 434
, behavior to gastic juice, 360
, behavior to pancreatic juice, 395
Cartilage gelatine, 433
Caseanic acid, 30, 33, 101
Caseid, 520
Casein, 36, 47, 100
, origin of, 537
, from woman's milk, 530
, from cow's milk, 518
, quantitative estimation of, 526
, absorption of, 413
, behavior towards rennin, 362, 520
, behavior to gastic juice, 47, 521, 530, 531
, heat of combustion, 724
Caseinokyrin, 58, 59
Caseinic acid, 30, 33, 101
Caseinogen, 521
Caseoses, 52, 522
, relation to the coagulation of blood, 171
Castor bean, 25
Castoreum, 692
Castorin, 692
Catalases, 7, 20. See also the fluids and tissues.
Catalyzers, 7, 14, 15, 20, 201
Catheterization of the lungs, 708, 709
Cat's milk, 527
Cell, animal, 139-169
Cell constituents, primary and secondary, 140
Cell fibrinogen, 271
Cell globulins, 141, 194
Cell membrane, 142, 360
Cell nucleus, 149
Cellulose, 129
, fermentation of, 129, 130, 397, 404
, solution of, in the cæcum, 398
Cement, (in tooth structure), 440
Cephalin, 485, 488
Cephalic acid, 485
Cephalopods, flesh of, 76, 455, 478
Cerebrin, 268, 480, 481 483, 484,
, in pus, 268
Cerebrinin phosphoric acid, 486
Cerebrinic acid, 486
Cerebron, 480, 483, 485
Cerebrosides, 194, 481, 482, 483

Cerebrospinal fluid, 264, 489
Cerolein, 138
Cerotic acid, 138
Cerumen, 692
Cervical ligament, 75, 76, 429
Cetaceans, bones of, 438
Cetin, 138
Cetyl alcohol, 138
Chalaza, 506
Charcot's crystals, 247, 496
"Charge theory," 364
Cheno-taurocholic acid, 315
Chief (adelomorphic) cells, 349, 364
Children's urine, 543, 549, 583
Chitaminic acid, 121
Chitaric acid, 121
Chitin, 81, 120, 686
, behavior in tryptic digestion, 395
Chitosamine, 120, 686. See also Glucosamine.
Chitosan, 687
Chitose, 121
Chloral hydrate, behavior in the animal body, 609, 632
, action upon the secretion of bile, 309
Chloral secretin, 309
Chlorates, poisoning with, 203, 648
Chlorazol, 32
Chlorbenzene, behavior in the animal body, 639
Chlorides, elimination by the urine, 616–619, 694, 695
, elimination by the sweat, 695
, action on protein metabolism, 754
, insufficient supply of, 736
. See also various fluids and tissues.
Chlorochrome, 282
Chlorocruorin, 219
Chloroform, action on the elimination of chlorides, 615
, action on the muscles, 466
, action upon proteins, 40
, behavior in the animal body, 631
Chlorometer, 618
Chlorophan, 491
Chlorophyll, 2
, relation to blood-pigments, 197, 214
Chlorosis, 246
Chlorphenylmercapturic acid, 639
Chlorrhodinic acid, 269
Chlortoluene, behavior in the animal body, 636
Cholagogues, 308
Cholamine, 315
Cholalic acid, 310, 315–318. See also Cholic acid.
Cholanic acid, 317
Cholecyanin, 323, 324
Choleic acid, 312, 317, 318
Choleprasin, 319, 324
Cholepyrrhin, 320
Cholera, blood in, 240
, sweat in, 694
, ptomaines in, 25
Cholera bacilii, behavior with gastric juice, 371
Cholestanol, 335
Cholestenone, 335
Cholesterilene, 334
Cholesterin, 334, 335
in blood-serum, 183, 194, 220
in the bile, 310, 326, 327, 328
in gall-stones, 333, 334
in the brain, 480, 487, 488
in the urine, 675, 682
, importance in the cell, 140, 143
, behavior toward saponin, 337
Cholesterin calculi, 334
Cholesterin ester in blood-serum, 183
Cholesterin fat, as protective fat, 691
Cholesterin-propionic ester, 335
Cholesterinic acid, 315
Cholesterone, 334
Choletelin, 322, 323
, relation to urobilin, 603
Cholic acids, 315, 316, 335
Cholic acid azide, 312, 315
Cholic acid hydrazide, 312
Cholic acid urethane, 315
Choline, 25, 145, 147, 264, 325, 394, 482
Cholohæmatin, 324
Choloidic acid, 319
Cholylic acid, 316
Chondrigen, 77, 430
Chondrin, 81, 269
Chondrin balls, 433
Chondro-albumoid, 433
Chondroitic acid, 431
Chondroitin, 431
Chondroitin-sulphuric acid, 42, 65, 66, 69, 70, 431
, in urine, 611, 647
, in kidneys, 542
Chondromucoid, 69, 430, 433
Chondroproteids, 65, 66, 69
in the urine, 647
Chondrosin from chondroitin-sulphuric acid, 431
from sponges, 69
Chorda saliva, 340
Choroid coat, 493
, pigment of, 688
Chromatin, 149
Chromhidrosis, 695
Chromogens in urine, 601
in suprarenal capsule, 277
Chrysophanic acid, action on urine, 639
Chyle, 250-252
Chyluria, 675
Chyme, 366
, investigation of, 373–376

Chymosin, 13, 16, 186, 361, 520
  , detection in gastric contents, 373
  , occurrence in the pancreas, 386, 395
  . See also Rennin.
Cilianic acid, 315
Cinnamic acid, behavior in the animal body, 585
Citric acid in milk, 518, 532
Clupeine, 63, 64
Coagulated proteids, 36, 61
Coagulation of the blood, 170, 171, 176–178, 225–235
  , intravascular, 234
  of milk, 362, 373, 396, 515, 519, 530
  of muscle-plasma, 448, 452, 453, 467
Coagulins, 232, 233
Coaguloses, 56
Cobra poison, 234
Coccinic acid, 690
Coccygeal glands, 692
Cochineal, 690
Cochinillic acid, 690
Codfish, eggs, 504
  , spermatozoa, 62
Coefficient, Häser's, 627
  , respiratory, 445, 472, 721, 731
  , dissociation, 190
  , extinction, 218
  , urotoxic, 615
Coffee, action on metabolism, 755
Collagen, 37, 76–78, 395, 428, 433, 435
Colloid, 68, 275, 499
Colloids, 39, 40
Colloid corpuscles, 499
Colloid cysts, 498
Colon, exclusion of, 427
Coloring-matters. See various pigments.
Colostrum, 528, 533
Colostrum corpuscles, 528
Comma bacillus, behavior with gastric juice, 371
Compound proteids, 36, 65–72
  . See also the different groups of protein substances.
Conalbumin, 507
Conchiolin, 37, 81, 82
Concentration, molecular. See various fluids.
Concrements. See various calculi.
Cones of the retina, pigment of, 491
Conglutin, calorific value of, 724
Connective tissues, 428–430
Copaiva balsam, action on the urine, 639
Copper in blood, 187, 239
  in bile, 310
  in biliary calculi, 334
  in hæmocyanin, 219
  in protein substances, 26
  in turacin, 690
Cornea, 434, 493
Cornein, 37, 81, 82
Cornicrystalline, 82
Corpora lutea, 216, 498
Corpse wax, 442
Corpulence, diet cures for, 779, 771
Corpus callosum, 488
Corpuscula amylacea, 486
Cow's milk, 515–529
  , general behavior, 515, 516
  , analysis of, 524–527
  , anti-putrefactive action of, 405, 588, 589
  , coagulation with rennin, 363, 373, 516, 530
  , behavior in the stomach, 530, 531
  , composition of, 526–528
Cream, 530
Creatine, relation to formation of urea, 455, 550
  , relation to muscular activity, 470
  , properties and occurrence, 455
Creatinine, relation to muscular activity, 470, 472, 563
  , properties and occurrences, 563, 564
  zinc chloride, 564
Cresol, 30, 401, 588, 589
Cresol-sulphuric acid, 588, 589
Crotonic acid, 674
Crude fibre, digestion of, 427
Cruor, 172
Crusocreatinine, 457
Crustaceorubin, 691
Crusta inflammatoria or phlogistica, 225
Crystalbumin, 493
Crystalfibrin, 493
Crystallins, 491, 492
Crystalline lens, 492, 493
Crystalline seralbumin, 181
Crystalloids, 39
Cumic acid, 634, 635
Cuminuric acid, 635
Curd, 442, 520
Cuorin, 144
Cyanhæmoglobin, 205
Cyanhydrines, 106
Cyanmethæmoglobin, 205
Cyanocrystalline, 509, 690
Cyanuric acid, 555, 568
Cyanurin, 601
Cyclopterine, 63
Cymene, 634
Cyprinine, 63
Cysteine, 28, 29, 33, 93, 94
  , conjugation in animal body, 639
Cysteinic acid, 93
Cystine, 28, 29, 34, 92, 93, 94, 331, 675
  , occurrence in urine, 611, 675, 676
  , occurrence in urinary sediments, 680
  , occurrence in urinary calculi, 682
  , occurrence in sweat, 695
  . behavior in animal body, 331, 675, 676

Cystinuria, 24, 92, 615, 675
Cysts, tapeworm, 265
, ovarial, 498, 502
, thyroid, 275
, mucoid substances of, 498-501
Cytin, 271
Cytoglobin, 36, 142, 228, 271
Cytosine, 152, 156, 165, 166
Cytotoxines, 186
Cytozym, 232

Damaluric acid, 616
Damolic acid, 616
Defibrinated blood, 172
Dehydrochloride hæmin, 212
Dehydrocholan, 311
Dehydrocholic acid, 315
Dehydrocholeic acid, 317
Dehydrocholesterin, 335
Delomorphoic or parietal cells, 349, 364
Denigè's reaction for uric acid, 576
Denigè's-Mörner's tyrosine test, 91
Dentin, 437, 440
Dermoid cyst, 502
Dermocerin, 691
Dermo olein, 691
Desaminoalbuminic acid, 49
Desaminoproteic acids, 31
Descemet's membrane, 69, 492
Desoxycholic acid, 316
Deuterocaseoses, 52
Deuteroelastose, 76
Deuteroproteose, 52, 60, 644
Deuterogelatose, 76
Deuteromyosinose, 79
Deuterovitellose, 52
Dextrins, 128
, formation from starch, 128, 344, 388
, loading the stomach with, 364
, occurrence in the gastric contents, 367
, occurrence in muscles, 459
, occurrence in portal blood, 242, 419
Dextrin-like substance in the urine, 608
Dextrose, 114-118
in blood, 184, 237, 242, 297-304
in urine, 297, 608, 654-665
in the lymph, 250
in muscles, 459
in the vitreous humor, 491
, preparation of, 118
, fermentation of, 20, 115, 657
, detection of, 118, 655-660
, reactions of, 115-117
, absorption of, 419
, quantitative estimation, 600-665
Diabetes mellitus, 297-306, 654
, elimination of ammonia by the urine in, 625
, relationship of the liver to, 300
Diabetes mellitus, relationship of the pancreas to, 301-303
, blood in, 240, 297
, amount of sugar in blood in, 297
, urine in, 543, 628, 654, 670
, CO_2 in the blood in, 701
, oxybutyric acid in the blood in, 702
, oxybutyric acid in the urine in, 625, 673
Diacetic acid. See aceto-acetic acid.
Dialuric acid, relationship to formation of uric acid, 573
Diamide, poisoning with, 584
Diamino acids, 33, 96-101
Diamines, 24, 97, 98
in the urine, 615, 676
in the intestinal contents, 24, 676
Diaminoacetic acid, 97
Diamino-caproic acid. See Lysine.
Diaminopropionic acid-dipeptide, 35
Diaminotrioxydodecanoic acid, 101
Diamino-valerianic acid, 97. See Ornithine.
Diastatic enzymes, 16, 185, 295, 343, 388. See also other enzymes.
Diastase in the blood, 185
Diazo reaction, Ehrlich's, 612, 613
Diazobenzol-sulphonic acid, reaction with sugar, 117
Dibenzoylornithin, 97
Dicalcium casein, 519
Dichlorpurine, 157
Diet cures, 770, 771
Diet for various classes of people, 763-765
Digestion, 339-427
Digestibility of food stuffs, 367-370, 417, 418, 421, 423, 425
Diglycyl-glycine, 35
Dileucyl-glycyl-glycine, 35
Dileucylcystine, 35
Dimethylaminobenzaldehyde, 43, 638, 675
Dimethylaminobenzoic acids, 638
Dimethyltoluidine, 638
Dimethylketone. See Acetone.
Dioxyacetone, 114
Dioxybenzenes, 591, 592, 633
Dioxydiaminosuberic acid, 33, 101
Dioxynaphthalene, 633
Dipalmitylolein, 132
Dipeptides, 35-37
, behavior with trypsin, 395, 396
Diphtheria toxins, action of the gastric juice upon, 371
Disaccharides, 123
in urine, 420, 665, 666
, inversion of, 123, 293, 360, 378, 419
as glycogen formers, 293, 294

Dissociation degree, 190
Dissociation coefficient, 190
Distearyllecithin, 143
Distearylpalmitin, 132
Doeglic acid, 135
Dog's milk, 529, 535
Dolphin's milk, 529
Donné's pus test, 651
Dotterplätchen, 37, 503, 509
Dropsical fluid, 262
Dulcite, 106
  , relation to formation of glycogen, 291
Dysproteose, 51
Dyslysins, 319
Dyspeptone, 359
Dyspnœa, action on protein catabolism, 548, 758

Ear, fluids of, 494
Earthy phosphates, elimination by the urine, 618, 619, 625, 626,
  , absorption of, 426
  , solubility in fluids rich in protein, 439
  , occurrence in bone-earths, 436-439
  , occurrence in calculi, 333, 411, 681, 682
  , occurrence in sediments, 678, 679
  . See also different earthy phosphates.
Ebstein's diet cure, 770, 771
Echinochrom, 219
Echinococcus cysts, cyst wall, 687
  , cyst contents, 265
Eck's fistula, 552
Edestan, 62
Edestin, 33, 46, 62, 100
  , absorption of, 413
Edible bird's nests, 69
Eel, flesh of, 475
Eel-serum, 171, 234
Egg, 502
  , hen's, 502-512
  , absorption in the intestine, 417
  , incubation of, 510-512
Egg albumin. See Ovalbumin.
Egg-shell, 73, 323, 509
Egg yolk, 502
Egg-white, 506
  , albumin of the, 507
Ehrlich's diazo reaction, 612, 613
  test for bile-pigments, 654
  glucosamine test, 121
  urine test, 674
Elaic acid, 134
Elaidic acid, 135
Elaidin, 135
Elastin proteoses, 76, 77
Elastin, 37, 75, 76, 100
  , behavior with gastric juice, 360
  , behavior with trypsin, 395

Elephant bones, 436
  milk, 529
  teeth, 440
Ellagic acid, 412
Embryo of the hen, development of, 511, 512
Emulsin, 13, 609
Emydin, 509
Enamel (of the teeth), 440
Encephalin, 482, 484
Endoenzymes, 22
Endolymph, 494
Energy, potential, of foodstuffs, 723-728
Enterokinase, 379, 383, 384, 386
Enzymes in general, 9-23
  , zymogens, 16
  . See various enzymes in the tissues, organs and fluids.
Epidermis, 73, 685
Epiguanine, 157, 578, 580
Epinephrin, 278
Episarkine, 157, 578, 580
Erepsin, 153
  , importance for absorption, 380, 391, 416
Erucic acid, 131
  , absorption of, 423
Erythyrite, relation to glycogen formation, 291
Erythrocytes, 191-196. See also red blood-corpuscles.
Erythrodextrin, 128, 344
Erythropsin. See Visual purple.
Esbach's method for estimating proteid in urine, 645.
Esters, cleavage of, 284, 389
  , synthesis of, 390
Ethal, 138
Ether, action on the blood, 193, 195
  , action upon proteins, 40
  , action on the secretion of gastric juice, 352
  , action on the muscles, 466
Ethereal sulphuric acids in the bile, 310, 325, 327
Ethereal sulphuric acids in the perspiration, 694
Ethereal sulphuric acids in the urine, 588-595, 622, 633, 637
Ethereal sulphuric acids, synthesis of, in the liver, 280
Ethyl alcohol, production by fermentation, 10, 11, 21, 302, 396, 461
  , production in the intestine, 400
  , passage of, into milk, 539
  , behavior in the animal body, 632, 754
  , action on the secretion of gastric juice, 351, 352, 365, 369
  , action on the pancreatic juice, 385
  , action on the muscles, 465, 466

Ethyl alcohol, action on metabolism, 754
, action on digestion, 369
, action on proteins, 40, 41
Ethyl benzene, behavior in the animal body, 634
Ethylene glycol, relation to formation of glycogen, 291
Ethylenimine. See Spermine.
Ethylidene-lactic acid, 460. See also other lactic acids.
Ethyl mercaptan, behavior in the animal body, 632
Ethyl-sulphuric acid, behavior in the animal body, 631
Ethyl sulphide, formation from protein, 28, 30, 33
, behavior in the animal body, 612
Euglobulin, 179, 180
Euxanthic acid, 122, 638
Euxanthon, 638
Euxanthon glucuronic acids, 610
Excrements, 408, 410, 717, 718
in dogs with biliary fistula, 407
Excreta, of the animal organism, 715–720
, division by the various channels, 717
Excretin, 410
Excretolic acid, 410
Exostosis, 438
Expectorations, 713, 714
Extinction coefficient, 218
Extracellular action of enzymes, 22
Exudates, 256–265
Eye, 489–494

Fæces. See Excrements.
Fat, origin in the body, 442–446
, general properties, detection and occurrence, 131–138
, relation to work, 471–474
to the formation of glycogen, 291, 292
, calorific value of, 723–728
, nutritive value of, 723–728, 747, 748, 751, 752
, rancidity of, 133,
, absorption of, 421–427
, behavior with gastric juice, 363, 364
, behavior with pancreatic juice, 388–390
, saponification of, 133, 389, 423
, action of, on the secretion of bile, 308
, action of, on the secretion of gastric juice, 349–351
, action of, on the secretion of pancreatic juice, 384, 385
, iodized, behavior of, in the animal body, 442, 538
, estimation of, 136, 137, 526, 527
, metabolism of, in activity and at rest, 471
, metabolism of, in starvation, 729
, metabolism of, with various foods, 740, 741, 770, 771
Fat, sugar formation from, 304–306
Fat formation, from proteins, 442–446
, from carbohydrates, 442–446
Fat-sweat, 692
Fatty acids, general properties, detection and occurrence, 131–138, 441
, solubility in bile, 422, 423
, absorption of, 422
, synthesis, 444, 445
to neutral fats, 421
Fatty degeneration, 443
Fatty infiltration, 282, 283, 443
Fatty series, behavior of members in the animal body, 629
Fatty tissue, 441, 446
, behavior with gastric juice, 360
Feathers, 73
, pigments of, 692
Fehling's solution, 116, 660
Fellic acid, 318
Fermentation, 10, 11, 20, 21, 109, 111, 115
in the intestine, 397, 399 403, 404
in the urine, 677, 678
in the gastric contents, 371, 373
. See also various fermentations, alcoholic, etc.
Fermentation lactic acid, properties, occurrence, etc., 460–462
in the gastric contents, 353
in the souring of milk, 460, 515, 516
, detection in the gastric contents, 374, 375
Fermentation saccharometer, 664
Fermentation saccharomanometer, 664
Fermentation test in the urine, 657, 663
Ferments in general, 9–23
inorganic, 14
. See various enzymes.
Ferratin, 282
Ferrine, 282
Fevers, elimination of ammonia in, 625
, elimination of uric acid in, 569
, elimination of urea in, 548
, elimination of potassium salts in, 623
, metabolism of proteins in, 548
Fibre, crude, utilization of, 427
Fibres, elastic, in sputum, 714
, reticulate, 428
Fibrin, 36, 171, 174, 183, 225, 228, 230
, occurrence in transudates, 260
, Henle's, 495
Fibrin coagulation, 175–178, 225–235
Fibrin calculi, 411, 682
Fibrin digestion, 356, 373, 392–394

Fibrin ferment, 16, 175, 176, 177, 183, 228–234
Fibrin formation, 175–178, 225–235
Fibrin globulin, 173, 175, 177, 183
Fibrin soluble. See Serglobulin.
Fibrinogen, 36, 172–177, 183, 228–230, 250
Fibrinolysis, 173, 175
Fibrinoplastic substance. See Serglobulin.
Fibroin, 37, 81, 82
Fischer-Weidel's reaction, 160
Fish-bones, 438
Fish-eggs, 37, 504, 509
Fish-scales, 81, 160
Fish, bile of, 310, 328
, spermatozoa of, 63, 151, 497
, swimming-bladder of, 160, 710
, visual purple of, 490
Fish-glue, 77
Flesh, metabolism, in starvation, 729
, metabolism, with various foods, 739–753
Flesh quotient, 476
Florence's sperma reaction, 496
Fluoride plasma, 231
Fluorine in bones, 436
in enamel, 440
Fly-maggots, formation of fat in, 443
Foods, influence on the secretion of intestinal juice, 377
, influence on the secretion of bile, 308
, influence on the secretion of gastric juice, 350, 351
, influence on the secretion of pancreatic juice, 382, 383
, influence on the secretion of milk, 536
, influence on the elimination of uric acid, 569
, influence on the elimination of urea, 548
, influence on the elimination of purine bases, 578
, influence on fæces, 408, 409, 417, 418, 421, 719,
, influence on metabolism, 762
, requirements, 763–771
, various, 739–753
, insufficient supply of, 734–739
Foodstuffs, necessity of, 715
combustion heat of, 723–728
Formaldehyde, formation in plants, 1, 114
, action upon proteins, 50
, combination with urea, 556
, relation to sugar formation, 113, 114
Formic acid in gastric contents, 376
, passage of, into urine, 607, 629
Frog's eggs, 509
membrane of, 66
Fructose. See Levulose.
Fruit sugar. See Levulose.
Fundus glands, 349, 363, 364
Fungi, glycogen in, 288
Fungi, tyrosinase in, 18
Fumaric acid, 32;
Furfuracrylic acid, 637
Furfuracryluric acid, 637
Furfurol, from pentoses, 111
, relation to Pettenkofer's bile-acid tests, 312
, reagent for urea, 555
, behavior in the animal body, 637
Fuscin, 491

Galactonic acid, 120
Galactose, 106, 120, 524
, from cerebrins, 484, 485
, relation to formation of glycogen, 293
, passage of, in the urine, 665
Galactosamine, 71, 121
Galactosides, 108
Gallacetophenon, behavior in the animal body, 638
Gallic acid, behavior in the animal body, 597, 637
Gallois's inosite test, 459
Galtose, 108
Gas, exchange of, in various ages, 755–757
, exchange of, through the skin, 695
, exchange of, in starvation, 730, 731
, exchange of, in various conditions of the body, 445, 446, 472, 720, 721, 730, 733, 757, 759, 761
, exchange of, in the muscles, 468, 472
, exchange of, starvation requirement of, 733
Gases of the blood, 696–702
of the intestine, 403, 404
of the bile, 329, 702
of the urine, 626, 702, 703, 712
of the hen's egg, 509, 510
of the lymph, 251, 702
of the muscles, 465, 468
of the transudates, 259, 712
of the stomach, 372
Gastric contents. See Chyme.
Gastric fistula, 350, 352
Gastric juice, 349
, composition of, 353, 354
, secretion of, 349–352
, estimation of acidity of, 373, 374, 376
, relation to intestinal putrefaction, 371, 406–408
, action of, 356–363, 366–371
Gastric lipase, 363
Gastric mucosa, 348
Gelatine, 28, 77–80, 100, 582
, relation to glycogen formation 291
, relation to coagulation of blood, 233
, putrefaction of, 401
, nutritive value of, 745
, behavior with gastric juice, 360
, behavior with pancreatic juice, 391, 395

Gelatine in the egg, 511
Gelatine and the detection of trypsin, 290
Gelatine-forming substances. See Collagen.
Gelatine peptones, 57, 58, 79
Gelatine sugar. See Glycocoll.
Gelatinous tissues, 429
Gelatoses, 79
  , relation to blood coagulation, 171
Generation, organs of, 495–513
Gentisic acid, 599
  , behavior in the animal body, 637
Gentisic aldehyde, 599
Gerhardt's diacetic acid reaction, 672
Globan, 46
Globin, 62, 99, 197, 208
Globulins, 36
  , general characteristics, 45
  , in starvation, 187
  , in urine, 643
  , in protoplasm, 140
  . See also the different globulins.
Globuloses, 52
Glucalanine, 81
Glucase, 21, 185, 345, 346
Glucocyanhydrin, 106
Glucoheptose, 106
Gluconic acid, 105, 114, 300
Glucosamine, 108, 120, 121
  from chitin, 120
  from proteins, 32, 65, 67, 500, 506–508
  in diabetes, 300
  relation to formation of glycogen, 294
Gluconucleoproteids, 71, 72
Glucoproteids, 36, 65–72, 141, 428, 430, 500, 506
  , relation to formation of glycogen, 292, 293, 295
Glucoproteose, 55
Glucosaminic acid, 121
Glucosan, 115
Glucose, 105, 106, 114. See also Dextrose.
Glucosides, 13, 108, 110
Glucosoxime, 106
Glucorone, 122
Glucothionic acid, 222, 272, 285, 431, 514, 542
Glucuronic acid, 121, 122
  , relation to formation of glycogen, 291
  , conjugated, 122, 609, 632, 638, 666
  in diabetes, 300
  in blood, 184
  in bile, 310, 325
  in urine, 609, 632, 638, 667
Glutamic acid, 88
Gluteines, 77
Gluten casein, 100
Gluten proteins, 100
Glutin peptones, 58, 79
Glutokyrin, 58
Glutose, 108
Glutinase, 391, 392
Glutinic acid, 78
Glyceric acid, 630
Glycerine aldehyde, 80
Glycerine relation to formation of glycogen, 291
  relation to formation of sugar, 305
Glycerophosphoric acid, 144, 247, 272, 277, 325
Glycerophosphoric acid in urine, 608, 614
Glyceroses, 114
Glycine. See Glycocoll.
Glycocholeic acid, 312
Glycocholic acid, 310, 312
  , occurrence in excrements, 404
  , occurrence in bile from various animals, 328
  , absorption of, 426
  , behavior to intestinal putrefaction, 406
Glycocholates from rodents, 313
Glycocoll, 83
Glycocoll, relation to formation of uric acid, 568, 572
  , relation to formation of urea, 550, 551, 630
  , synthesis with, 3, 585, 586, 635
Glycogen, 140, 287–307
  , origin of, 289–307
  , relation to muscular activity, 468, 472
  , relation to muscle rigor, 466
  , relation to pepsin secretion, 365
  , occurrence in sputum, 714
  , occurrence in leucocytes, 221
  , occurrence in the lungs, 713
  , occurrence in the lymph, 251
  , occurrence in protoplasm, 140, 148, 221, 268
Glycol aldehyde, 2
Glycolysis, 185, 302, 303, 396
Glycolytic enzyme, 20, 185, 302, 303
Glycosuria, 297–307
  , alimentary, 298, 420
Glycosuric acid, 597
Glycuronic acid, 121. See Glucuronic acid.
Glycylalanine, 35, 395
Glycylglycine, 35, 396, 630
Glycyl-l-leucine, 35
Glycyl-l-tyrosine, 35
Glyoxyl diureide. See Allantoin.
Glyoxylic acid, as reagent, 42
Gmelin's test for bile-pigments, 321
  test for bile-pigments in urine, 652
Goat's milk, 528, 529
Gold equivalent of the proteins, 41
Goose-fat, absorption of, 423

Gorgonin, 82
Gout, elimination of uric acid in, 570
Graafian follicles, 498
Grape-moles, 512
Grape-sugar. See Dextrose.
Guaiaconic acid ozonide, 649
Guaiacum blood test, 648
Guanase, 16, 271, 273, 571
Guanidine, 32, 33, 501, 550
Guanine, 158, 160
in urine, 578
Guanine gout, 160
Guano, 160, 569
Guano-bile acids, 313
Guanovulit, 510
Guanylic acid, 151, 153, 155, 156, 381
Gulonic acid lactone, 121
Gulose, 113, 118
Gums, various, 129
, animal, 67
, animal, in urine, 608
Gunning-Lieben's acetone reaction, 670
Günzberg's reagent for free HCl, 374

Hæmagglutination, 195
Hæmase, 20
Hæmataërometer, 706
Hæmatin, 197, 209
, relation to bilirubin, 332
, relation to urobilin, 603
, neutral hæmatin, 208
Hæmatinogen, 216
Hæmatinometer, 217
Hæmatinic acids, 210, 211, 325
Hæmatinic acid imide, 211, 320
Hæmatinic acid ester, 211
Hæmatocrit, 236
Hæmatogen, 503, 510
Hæmatoglobulin. See Oxyhæmoglobin.
Hæmatoidin, 216
, relation to bilirubin, 216, 320, 331
, occurrence in sputum, 714
, occurrence in corpora lutea, 498
, occurrence in excrements, 409
, occurrence in sediments, 680
Hæmatoporphyrin, 213
, relation to chlorophyl, 197, 214
, relation to bilirubin, 215, 320, 332
, relation to urobilin, 214, 332, 603
, occurrence in urine, 601, 649, 650
, occurrence in lower animals, 690
Hæmatoscope, 219
Hæmaturia, 648
Hæmerythrin, 219
Hæmin, 211, 649
Hæmin crystals, 211, 649
Hæmochrom, 196, 199
Hæmochromogen, 197, 208, 209
, occurrence in muscles, 451
Hæmocyanin, 219
Hæmoglobin, 36, 65, 197
, composition of, 198
, properties and behavior, 202
, quantity in blood, 197, 242-247
, quantitative estimation, 217-219
. See also Oxyhæmoglobin and the combinations of hæmoglobin with other gases.
Hæmoglobinuria, 648
Hæmolysis, 193, 337
Hæmolysins, 186, 193
Hæmometer, 219
Hæmopyrrol, 197, 214
Hæmorrhodin, 207
Hæmoverdin, 208
Häser's coefficient,
Hair, 73, 685
, pigments of, 689
Hair-balls, 411
Half-rotation, 109
Hammarsten's reaction for bile-pigments, 322, 653
Haptogen-membrane, 517
Heat, action of, on metabolism, 761
of combustion of various foodstuffs, 723-727
Helicoproteid, 71
Heller's albumin test, 41
albumin test applied to urine, 640
Heller-Teichmann's blood test, 649
Hemicelluloses, 130
Hemicollin, 79
Hemielastin, 76
Hemiindigotin, 594
Heminucleic acid, 154
Hemipeptone, 51
Hemp-seed calculi, 681
Hen, development of the embryo of, 511, 512
Hen's egg, 502-512
, incubation of, 510
white of the, 506-510
yoke of the, 502, 506
Heptoses, 105
Herring, spermatozoa of, 63, 498
Heterolysis, 23
Heteroproteoses, 51, 55, 56, 57
Heterosyntonose, 100
Heteroxanthine, 157
in urine, 579
Hexone bases, 63, 96-100
Hexoses, 113-120
. See also the various hexoses.
High altitude, action on the blood, 245
Hippokoprosterin, 337
Hippomelanin, 688
Hippuric acid, 585
,properties and reactions, 587

Hippuric acid, estimation of, 578
, formation in the body, 3, 585, 635
, cleavage of, 4, 584, 587, 588
, occurrence of, 585
as sediment, 679, 680
Hirudin, 231, 233
Histidine, 63, 99, 100, 108
Histidylhistidine, 35
Histone, 27, 36, 61, 100, 208, 229, 270
in urine, 647
Histozyme, 588
Hofmann's tyrosine test, 91
Hog-fat, 423
, absorption of
Hog-flesh, 475
Holothuria, mucin of, 69
Holozyme, 232
Homocerebrin, 482–484
Homogentistic acid, 68, 90, 597–599
Hoppe-Seyler's CO blood test, 206
xanthine test, 160 .
Horn, 73, 685
Horn substance. See Keratins.
Horse's milk, 529
Human milk, 529–535
, behavior in the stomach, 530, 531
Humin substances in urine, 600
Humor, aqueous, 264
, vitreous, 491
Huppert's reaction for bile-pigments, 322
reaction for bile-pigments in urine, 653
Hyalines, 68
of the walls of hydatid cysts, 687
of Rovida's substance, 141, 221, 267
Hyalogens, 65, 68
Hyalomucoid, 490
Hydatid cysts, 687
Hydræmia, 246
Hydramnion, 513
Hydrazones, 107
Hydrobilirubin, 320
, relation to urobilin, 603
Hydrocele fluids, 259, 264
Hydrocephalus fluid, 264
Hydroquinone, 591, 639
Hydroquinone sulphuric acid, 588, 592
Hydrochloric acid, secretion in stomach, 353, 364, 365, 373
, anti-fermentive action of, 371
, action of, on secretion of pancreatic juice, 384
, action of, on secretion of bile, 308, 309
, action of, on pylorus, 367
, material of, 371
, quantitative estimation in gastric contents, 375, 376
Hydrochloric acid, reagents for free HCl in gastric contents, 374
Hydrocinnamic acid, behavior in the animal body, 585
Hydrocyanic acid, action on peptic digestion, 359
, action on tryptic digestion, 393
Hydrogen in putrefactive and fermentive processes, 5, 401, 403
Hydrogen peroxide, decomposition of, by catalases, 7, 20
Hydrogenases, 19
Hydrolytic cleavages, 9, 16
. See also the various cleavages.
Hydronephrosis fluid, 542
Hydroparacoumaric acid, 597
, in intestinal putrefaction, 401
Hydroxylamine, poisoning with, 584
Hyoglycocholic acid, 313
Hyperglucæmia, 298, 299
Hyperisotonic solutions, 193
Hypisotonic solutions, 193
Hypnotics, relation to formation of glycogen, 291
Hypogæic acid, 138
Hyposulphites in the urine, 612
Hyposulphurous acid in urine, 612, 631
Hypoxanthine, 158
, properties, 161, 162
, passage of, into urine, 578

Ichthidin, 504, 509
Ichthin, 509
Ichthulin, 71, 504, 509
Ichthylepidin, 81
Ignotin, 457
Icterus, 307, 333, 652
, urine in,
Immunity, 25, 186
Incubation of the egg, 510
Indican test, Jaffé's, 594
, Obermeyer's, 594
Indican, urine, 592–594
, elimination in starvation, 404, 592, 593
, elimination in disease, 592, 593
Indigo, 402, 592, 594
, in sweat, 695
, in urinary sediments, 680
Indigo blue. See Indigo.
Indigo red, 594
Indigo sulphonic acid, 595
Indol, properties, 402
, formation from protein, 30, 34, 102
, formation in putrefaction, 401, 402, 588, 592
, formation from melanins, 689
, in the blood, 186
Indolacetic acid, 596
Indolaminopropionic acid, 30 102
Indophenol reaction, 18

Indoxyl. See Indol.
Indoxyl-glucuronic acid, 592, 595
Indoxyl red, 594
Indoxyl-sulphuric acid, 592, 595
Inosinic acid, 155, 454, 457
Inosite, properties and occurrence, 458, 459
in urine, 668
, relation to formation of glycogen, 291
Integral factor, 574
Intestinal calculi, 410–412
Intestinal fistula, 377, 380, 399
Intestinal gases, 403, 404
Intestinal juice, 377–379
Intestinal mucosa, 377
Intestine, putrefactive processes in, 399–408, 586–590
, reaction in, 399, 400, 406–408
, absorption in, 406, 411–427
, digestive processes in, 396–405
Intestine nucleic acid, 152
Intracellular enzymes, 22. See also the various organs.
Inulin, 127
, relation to formation of glycogen, 290
, relation to the secretion of pepsin, 365
Inversion, 123, 361, 397, 419
Invertases, 16, 185, 361, 378, 419
Invert-sugar, 123
Iodides and secretion of gastric juice, 364
Iodine equivalent, 137
Iodine, passage of, into milk, 539
, passage of, into sweat, 695
, passage of, into saliva, 347
, action upon protein, 30
, in the blood, 187, 243
, in glands, 27, 271, 275, 276
Iodized proteins, 30, 81, 275, 598
Iodized fats, 442, 538
Iodoform, behavior in the animal body, 631
test, Gunning's, 670
test, Lieben's, 670
Iodogorgonic acid, 82
Iodohæmatin, 213
Iodospongin, 81
Iodothyreoglobulin, 275
Iodothyrin, 275, 276
Ion action, 15, 39, 168, 169, 359, 393, 464
Iron in blood, 238, 239
in urine, 626
in new-born, 286, 535
, elimination of, 325, 332, 347, 626
and blood formation, 244, 245, 503
and bile formation, 332
, absorption of, 244, 245
. See also various tissues and fluids
Iron starvation, 738
Isobilianic acid, 315
Isocholanic acid, 317
Isocasein, 520
Isocholesterin, 335, 337, 691, 692
Isocreatinine, 454
Isocysteine, 93
Isodynamic law, 726
Isoglucosamine, 108
Isolactose, 16
Isoleucine, 87
Isomaltose, 125, 184, 344, 388
in urine, 608
Isosaccharin, relation to formation of glycogen, 291
Isoserine, 96
Isotonic solutions, 193
Isotropous substance, 447
Ivory, 440

Jaffé's indican test, 594
creatinine test, 566
Janthinin, 691
Japanese, nutrition of, 764
Jaune indien, 122
Jecorin, 147, 273, 283
, in blood, 184
Jequirity bean, 25
Jolles's reaction for bile-pigments, 653

Kathæmoglobin, 208
Kephir, 524, 528
, anti-putrefactive action, 405
Kephir lactase, 524, 610
Kerasin, 482, 283, 484, 488
Keratose, 73
Keratins, 37, 73–75, 685
, behavior in the stomach, 360
, behavior with pancreatic juice, 396
Ketones, behavior in the animal body, 632, 637, 638
Ketoses, 105, 113, 119
Kidneys, 541
, relation to formation of urea, 553
, relation to formation of hippuric acid, 586, 587
Kinases, 16, 176, 231, 232, 233, 379, 383, 386
Kjeldahl's method of determining nitrogen, 556
Knapp's titration method, 662, 663
Knee-joint cartilage, 364–434
Knop-Hüfner's method for determining urea, 562
Koprosterin, 335, 337, 408
Krinosin, 486
Kumyss, 524, 528
Kyestein, 680
Kynurenic acid, 597, 600
Kyrins, 58, 59
Kyroprotic acids, 31

Laborer, diet of, 763–770
Laccase, 18
Lactacidase, 21
Lactalbumin, 36, 522, 523, 526
Lactase, 524
in the intestine, 379, 419
in the pancreas, 386
Lactates. See Lactic acids, also 461–463

Lactic-acid fermentation, 110, 116, 371, 373, 389, 400, 460, 516, 524
in intestine, 397 399
in stomach, 371, 373
in milk, 515, 516, 523, 524
Lactic acids, 460
, in intestine, 397, 400
, in urine, 460, 572, 608
, in bones, 439
, in stomach, 353, 374
, relation to formation of uric acid, 572, 573
. See also Paralactic and Fermentation lactic acids.
Lacto-caramel, 524
Lacto-globulin, 522
Lactolase, 21
Lactone of saccharic acid, 121
Lactones of varieties of sugars, 105, 106
Lactophosphocarnic acid, 523
Lactoprotein, 523
Lactose. See Milk-sugar.
Laiose, 665
Lakey color of blood, 225
Lamb, intestinal fluid in the, 377
Lanoceric acid, 692
Lanocerin, 692
Lanolin, 337, 642
Lanopalmitic acid, 692
Lanugo hair, 512
Lard, absorption of, 424
Large intestine, extirpation of, 426, 427
, secretion of, 380
Latebra, 502
Lauric acid, 131, 138, 518
Lead in the blood, 239
in the liver, 286
passage of, into milk, 539
Lecithalbumins, 47, 349, 541
, relation to secretion of gastric juice, 349
, relation to secretion of urine, 541
Lecithans, 488
Lecithins, 143, 144
, in egg-yolk, 502, 503
, in the brain, 480, 488
, in the muscles, 463
, in milk, 518, 532
, in the liver, 283
, importance for cells, 143, 193
, putrefaction of, 146, 177, 404
Legal's acetone reaction, 671
Lens, (see Crystalline lens), 492
, capsule of, 69, 492
, fibres of, 492
Leo's sugar, 665
Lepidoporphyrin, 690
Lepidotic acid, 690
Lethal, 138
Leucæmia, blood, 158, 247
, uric acid, elimination in, 274, 569, 570
, purine bases in, 158, 247, 578
Leucin, 85–87
, relation to formation of uric acid, 572
, relation to formation of urea, 550, 551, 630
, passage of, into urine, 614, 675
, behavior in the animal body, 550, 551, 630
Leucin ester, 86, 87
Leucin ethylester, 87
Leucinic acids, 86
Leucinimide, 87
Leucocytes, relation to absorption, 416
, relation to formation of uric acid, 570
, in thymus gland, 272
Leucomaines, 24
, in urine, 615
, in muscles, 457
Leuconuclein, 229, 270
Leucylalanyl-glycine, 35
Leucyl-l-tyrosine, 35
Levolactic acid, 460
Levuline, 127
Levulinic acid, 67, 113, 152, 524
Levulose, 106, 108, 118, 119
, in urine, 664
, in blood, 184
, relation to glycogen formation, 293
, absorption of, 419, 420
, behavior in diabetics, 300
, in transudates, 260, 512
Lichenin, 127
Lieben's acetone reaction, 670
Lieberkühn's, alkali-albuminate, 48
, glands, 377
Liebermann's reaction for proteins, 43
Liebermann-Burchard's reaction for cholesterin, 336
Liebig's titration method for estimating urea, 557–560
Lienases, 273
Ligamentum nuchæ, 75, 76, 429
Lignin, 129
Linoleic acid, 131, 135
Linolic acid, 504
Linseed-oil, feeding with, 442, 538
Lion's urine, 567
Lipanin, absorption of, 423
Lipase, 16
in blood, 185
in stomach, 363
in the intestine, 379, 380
in the liver, 284
in pancreatic juice, 384, 388
in milk, 523
Lipiawsky's acetoacetic acid reaction, 672

Lipochromes, 186, 505
Lipoids, 193
Lipuria, 675
Lithium, in blood, 166, 239
Lithium lactate, 463
Lithium urate, 575
Lithobilic acid, 411
Lithofellic acid, 318, 411
Lithuric acid, 616
Liver, 280–287
, relation to coagulation of blood, 172, 234, 235
, relation to formation of uric acid, 571, 572, 573
, relation to formation of urea, 550, 551, 552, 554
, blood of, 242, 296, 297
, proteids of, 286, 287
, fat of, 282, 283
, quantity of sugar in, 295, 296, 297
Liver atrophy, acute yellow, 23, 284
, elimination of amino acids in, 284, 285, 675
, elimination of ammonia in, 554
, elimination of urea in, 554
, elimination of lactic acid in, 461, 608, 609
, autolysis, 23, 284
Liver cirrhosis, ascitic fluid in, 262, 263
, action of, on the elimination of ammonia and urea, 554
Liver extirpation, elimination of ammonia with, 554, 572
, elimination of uric acid with, 572, 573
, elimination of urea with, 554
, elimination of lactic acids with, 460, 572, 608
, action on formation of bile, 330
Lotahistone, 62
Lung catheter, 708
Lungs, 703, 704, 713, 714
Luteins, 505
in corpora lutea, 216, 498
, egg-yolk, 505
in blood-serum, 186
, relation to hæmatoidin, 216, 505
Lymph, 250-256
Lymphagogues, 255
Lymphatic glands, 269
Lymph-cells, quantitative composition of, 272
. See also Leucocytes.
Lymph-fibrinogen. See Tissue-fibrinogen.
Lysalbinic acid, 49
Lysatine and lysatinine, 99
Lysine, 33, 64, 98, 99, 186
Lysines, 25, 186, 193
Lysuric acid, 98
Lysyllysine, 35
Mackerel, flesh of, 475
, sperm of, 62, 63
Madder, feeding with, 438
Magnesium in urine, 625, 628
in bones, 436, 440
in muscles, 464, 475, 477
. See also various tissues and fluids.
Magnesium phosphate in intestinal calculi, 411
in urine, 619, 625, 628
in urinary calculi, 680, 681, 684
in urinary sediments, 677, 679
in bones, 436, 440
Magnesium soaps in excrements, 408
Malic acid, behavior in the animal body, 544
Maltase, 16, 125, 345, 346, 388
Maltodextrin, 128
Maltoglucase, 21, 185, 344, 346
Maltose, 123, 124
, formation from starch, 124, 127, 344, 388
, absorption of, 419
, relation to glycogen formation, 293
in intestine, 397, 419
, occurrence in urine, 667
Mammary glands, 514, 537
Mandelic acid, 634
Man in poorhouse, diet of, 770
Mannite, 106
, relation to formation of glycogen, 291
Mannonic acid, 114
Mannose, 108, 113, 114, 118
Mare's milk, 530
Margarine and margaric acid, 134
Marsh-gas, formation in putrefaction, 30, 401, 404
Martamic acid, 154. See also Methane.
Maschke's creatinine reaction, 565
Meat extracts, action on secretion of gastric juice, 365
, constituents of, 454, 456
Meat, utilization in intestinal tract, 417
, calorific value of, 724–726
, digestibility of, 368
, composition of, 443, 444, 474–476
. See also muscles.
Meconium, 410
Medulla oblongata, 486
Melanins, 154, 688–690
in the eye, 491
in the urine, 651
Melanogen in the urine, 651
Melanoidic acid, 688
Melanoidins, 27, 30, 154
Melanotic sarcoma, pigment of, 688
Melissyl alcohol, 138
Membranins, 69, 434, 492
Menstrual blood, 187, 242

Menthol, behavior in the animal body, 638
Mercaptan, from proteins, 30, 33, 73, 401
Mercapturic acids, 638
Mercury salts, passage of, into milk, 539
, passage of, into sweat, 695
, action on ptyalin, 346
, action on trypsin, 393
Mesitylene, behavior in the animal body, 635
Mesitylenic acid, 635
Mesitylenuric acid, 635
Mesoporphyrin, 214
Metacasein reaction, 521
Metabolism, dependence of external temperature upon, 761, 762
in various ages, 755-758
in work and rest, 467-474, 758-761
in different sexes, 756
in starvation, 728-733
with different foodstuffs, 739-754
in sleep and waking, 761
calculation of extent of, 720, 723
Metalbumin, 499, 500
Metallic sols, 14
Metaphosphoric acid, as reagent for proteins, 41, 642
Metazym, 232
Methæmoglobin, 203, 218
in urine, 648
Methal, 138
Methane, formation in putrefaction, 30 401, 404
Methose, 114
Methylenitan, 114
Methylethylmaleic acid anhydride, 211
Methylfurfurol, 336
Methyl glycocoll. See Sarcosin.
Methylguanidine, 455, 457, 565
Methylguanidin-acetic acid. See Creatine.
Methylhydantoic acid, 631
Methyl indol. See Skatol.
Methylimidazol, 108
Methyl mercaptan in proteins, 30, 401, 404
Methyl pentose. See Rhamnose.
Methyl pyridine, behavior in the animal body, 634, 637
Methyl-pyridyl-ammonium hydroxide, 639
Methylthiophene, 336
Methyluramine, 455, 457, 565
Methyl xanthine, 157, 579,
Micrococcus restituens, 415
Micrococcus urea, 677
Micro-organisms in intestinal tract, 24, 371, 398, 399, 405, 408
Milk, 515-542
, secretion of, 536, 537
, consumption of, in intestine, 417, 425, 530, 531
, blue, 540
Milk, anti-putrefactive action in intestine, 405, 589
in disease, 539
, passage of foreign bodies into, 538
, behavior in the stomach, 367, 371, 530, 531
. See also various kinds of milk.
Milk-fat, 517, 530
, formation of, 537, 538
Milk-globules from cow's milk, 516, 517
from human milk, 530
Milk-plasma, 518
Milk-sugar, 135, 524
, relation to formation of glycogen, 293
, properties of, 524,
, fermentation of, 524
, calorific value of, 724
, quantitative estimation of, 527
, absorption of, 419
, passage of, into urine, 293, 420, 524, 665
, origin of, 538
Millon's reagent, 42, 43
Mineral acids, alkali-removing action of, 544, 624
, action on the elimination of ammonia, 544, 624
Mineral bodies, elimination in starvation, 620, 623, 731
, insufficient supply of, 734-738.
See also the various fluids, tissues, and juices.
Modified proteins, 40
Molisch's sugar test, 117
Monamino acids, 83-96
behavior in animal body, 305, 462, 550, 572, 614, 630
Monosaccharides, 105-123
Moore's sugar test, 115
Mörner-Sjöqvist's method of estimating urea, 561
method of estimating acidity, 376
Mörner's tyrosine test, 89. See also Denigé's.
Morphine, passage of, into urine, 608, 639
, passage of, into milk, 539
Mucic acid, 120, 128, 524
, relation to formation of glycogen, 291
Mucilages, vegetable, 129
Mucin, 36, 65-68
in sputum, 714
in cysts, 501
in urine, 615, 647
in salivary glands, 339, 340
Mucin-like substances in bile, 309, 310, 329
in urine, 615, 646

Mucin-like substances in kidneys, 541, 542
Mucinogen, 66, 340, 509
Mucinoids. See Mucoids.
Mucin peptone, 67, 360
Mucoids, 36, 65, 68
    in ascitic fluids, 262
    in the vitreous humor, 491
    in the cornea, 434
    in connective tissue, 428–430, 435
    in the hen's egg, 506, 508
    in cysts, 498–501
Mucoproteoses, 360
Mucous glands, 66, 339, 348, 380
Mucous membranes of the stomach, 348
Mucous tissue, 430
Mucus of the bile, 310, 327
    of the urine, 542, 615, 647
    of synovial fluid, 265
Mulberry calculi, 681
Murexide test, 575
Muscle, coagulation of. See Muscle-plasma.
    , chemical tones of, 467
    , permeability of, 465
Muscle-fibres, 447, 465
    , permeability, 465
Muscle-pigments, 453
Muscle-plasma, 448, 449, 452, 453
    , coagulation of, 448, 452, 453, 466, 477
Muscle rigor, 465
Muscle-serum, 448
Muscle-stroma, 451
Muscle-sugar, 459
Muscle-syntonin, 451
Muscles, Bowman's disks, 448
    , non-striated, 477
    , striated, 447–477
    , blood of, 242, 468
    , chemical processes in work and rest, 467–474, 759
    , chemical processes in rigor, 465
    , proteins of, 447–454, 466, 470
    , extractives of, 453–464
    , enzymes of, 453
    , pigments of, 453
    , fat of, 463, 472–474
    , gases of, 465, 468
    , calorific value of, 725–727
    , mineral bodies of, 464, 477
    , amount of water in, 476
    , composition of, 474–477
Muscular energy, origin of, 472–474
Musculamine, 457
Muscular force, chemical processes in muscles, 467–474
    , action of, on urine, 544, 564, 567, 608, 616
    , action of, on metabolism, 470–474, 758–761
Musculin, 450, 452, 478
Mussels, glycogen of, 287
    , muscles of, 477
Mutton-fat, feeding with, 442
Mutton-fat. absorption of, 423, 424
Myeline forms. 481
Myelines, 481, 488
Myoalbumin, 449, 451
Myogen, 453
Myogen fibrin, 449, 453, 466
Myoglobulin, 449, 451
Myohæmatin, 454
Myoproteid, 452, 453
Myosin, 36, 221, 449, 450, 452, 466
    , absorption of, 413
Myosin ferment, 452, 453
Myosin fibrin, 449, 452
Myosinogen, 452
Myosinoses, 52
Myricin, 138
Myricyl alcohol, 138
Myristic acid in animal fat, 131
    in butter, 518
    in bile, 325
    in wool-fat, 692
Mytolin, 451
Myxœdema, 276
Myxoid cysts, 498

Nails, 73, 685
Naphthalene, action on urine, 639
    , behavior in the animal body, 633
Naphthalene sulphochloride as reagent, 675
Napthalene sulpho derivatives of amino acids, 92, 675
Napthol-glucuronic acid, 639
Napthol, reagent for sugar, 117, 659
    , behavior in the animal body, 609, 639
Napthyl isocyanate compound of the amino-acids, 92
Narcotics, relation to glycogen formation, 291
Native proteins, 40
Navel cord, mucin of, 66, 67, 429
Negative phase, 234
Neosine, 457
Neossin, 69
Neozym, 232
Nerves, 479, 480, 488
Neuridine, 481, 486, 502
Neurine, 145, 277, 485, 615
Neurochitin, 489
Neuroglia, 480
Neurokeratin, 73, 480, 488, 489
Neutral fats. See Fats.
Nicotine action upon the gases of the stomach, 372
Nitrates in the urine, 623
Nitric-oxide hæmoglobin, 207
Nitriles, behavior in the animal body, 631
Nitro-benzaldehyde, behavior in the animal body, 636
Nitro-benzene, 635
Nitro-benzoic acid, 636
Nitro-benzyl alcohol, 638
Nitro-cellulose, 130

Nitro-hippuric acid, 636
Nitro-phenol, 635
Nitro-phenyl-propiolic acid, reagent for sugar, 117, 659
  , behavior in the animal body, 592, 595
Nitro-toluene sulpho-compounds of amino acids, 92
Nitro-tolulene, behavior in the animal body, 638
Nitrogen, combined, quantity of, in intestinal evacuations, 717, 718
  , in meat, 443, 476
  , in protein bodies, 27
  , in urine, 548, 549
  , estimation of, in urine, 556–561
Nitrogen deficit, 718
Nitrogen elimination in work and rest, 470–473, 758, 759
  in starvation, 728–730
  with various foods, 739–752
  through the intestine, 417, 418, 717, 718
  through the urine, 548, 549, 611, 612, 620, 622, 717–719
  through the epidermis, 718
  through the sweat, 694, 718
  , relation to the elimination of phosphoric acid, 620
  , relation to the elimination of sulphuric acid, 622, 719
  , relation to digestive activity, 624, 717, 718, 762
Nitrogen, free, in blood, 696
  , in intestine, 403
  , in stomach, 372
  , in secretions, 702
  , in transudates, 702
  , in urine, 626
Nitrogen, residual, 183
Nitrogenous equilibrium, 718. See also Chap. XVIII
Nitroso-indol nitrate, 402
Non-striated muscles, 477
Non-biuret giving products, 54, 394, 417
Norisosaccharic acid, 121, 504
Novain, 457
Nubecula, 542, 615
Nucleases, 153, 271, 391
Nucleic acids, 71, 150–151, 156, 195
  in the urine, 647
Nuclein bases, 156–164
  in blood, 158, 186
  in the urine, 578
Nucleins, 72, 149
  , relation to elimination of alloxuric bases, 578
  , relation to formation of uric acid, 570, 571
  , relation to elimination of P_2O_5, 619, 620
  , behavior with gastric juice, 72, 149, 150, 350
  , behavior with pancreatic juice, 394, 395
Nuclein plates, 222
Nucleoalbumins, 36, 46, 141, 150
  in the bile, 310, 329
  in the liver, 281
  in the urine, 646
  in the kidneys, 541, 542
  in protoplasm, 141
  in transudates, 258, 261
  , behavior in pepsin digestion, 46, 150, 522, 532
Nucleoglucoproteids, 71, 72
Nucleohistone, 62, 221, 269
  , relation to coagulation of blood, 229
  in urine, 647
Nucleoproteids, 36, 71, 72, 142, 149
  in the liver, 282
  in gastric juice, 354, 355
  in blood, 172, 178
  in bile, 329
  in mammary glands, 514
  in muscles, 451, 477
  in the kidneys, 541
  in the pancreas, 149, 150, 381
  in protoplasm, 142
  in cell nucleus, 142, 149
  in thyroid gland, 275
  in thymus, 270
  , behavior in pepsin digestion, 71, 150
  , behavior with pancreatic juice, 395
Nucleotin, 154
Nucleotin phosphoric acid, 154
Nucleon, 457, 477
  in milk, 523, 532
Nucleosin, 165
Nutrition requirements, 763–771
  , of man, 739–753
Nylander's reagent. See Almén-Böttger's sugar test.

Obermeyer's indican test, 594
Obermüller's cholesterin reaction, 337
Oblitin, 457
Odoriferous bodies in the urine, 586
Œdema, subcutaneous, fluid from, 265
Oertel's diet cure, for corpulency, 770, 771

Œsophageal fistula, 350
Oleic acid, 135
Olein, 134
Oleodistearin, 132
Oligæmia, 246
Oligocythæmia, 246
Oliguria, 628
Olive oil, absorption of, 423
  , action on the secretion of bile, 308, 309
Onuphin, 69
Oocyanin, 509
Oorodein, 509
Opalisin, 523, 531
Opium, passage of, into the milk, 539
Optograms, 491
Orcin test, 111, 666
Organic acids, behavior in the animal body, 624, 629–631
Organized proteids, 742, 743
Organs, distribution of the blood in, 249
  loss of weight in starvation, 732
Organs of generation, 495–513
Ornithine, 97, 635
Ornithuric acid, 97, 635
Orotic acid, 523
Orthonitrophenylpropiolic acid. See Nitrophenylpropiolic acid.
Orylic acid, 523
Osaminic acid, 107
Osamines of varieties of sugar, 107
Osazones, 107
Osmosis, relation to absorption, 427
  , relation to lymph formation, 255, 256
Osmotic pressure of blood, 189, 159
  of urine, 546
Osones, 107
Ossein, 76, 435
Osseoalbumoid, 435
Osseomucoid, 66, 435
Osteomalacia, 438, 439
Osteoporosis. See Osteosclerosis.
Osteosclerosis, 438
Otoliths, 494
Ovalbumin, 32, 507
  , relation to glycogen formation, 290
Ovarian cysts, 498–502
Ovaries, 498
Ovoglobulin, 33, 506
Ovomucoid, 68, 508
Ovomucin, 506
Ovovitellin, 36, 503
Ovum, 502–512
Oxalate calculi, 680, 681
Oxalate of lime. See Calcium oxalate.
Oxalates, action on blood coagulation, 171, 225
Oxalic acid, in the urine, 582, 583, 678, 680
  behavior in the animal body 582, 783, 629
Oxaluric acid, 568, 582
Oxaluric-acid amide, 32
Oxamide, 29, 32
Oxaminic acid, 32
Oxidases, 8, 16–19, 185
  . See also the tissues and fluids.
Oxidation ferment. See Oxidases.
Oxidations, 3–9, 17–19, 629–631, 633, 703
  in diabetes, 300
Oximes, 106
Oxonic acid, 568
Oxyacids, formation in putrefaction, 30, 401
  , detection of, 597
  , passage of, in urine, 401, 596
  , in the sweat, 694
Oxybenzoic acid, behavior in the animal body, 635, 636
Oxybenzenes, 633
Oxbutyric acid, 668, 672, 673
  , detection and estimation, 673–675
  in the blood, 702
  , passage of, into the urine, 625, 668, 669, 672, 673
Oxydiaminosuberic acid, 29, 33
Oxydiaminosebacic acid, 282
Oxyethylsulphonic acid, behavior in the animal body, 632
Oxyfatty acids in animal fat, 131
Oxygen, consumption, 199, 704, 705
  in work and rest, 468, 472
  in starvation, 729. 730
  through the skin, 695
Oxygen, activity of, 3–8
  in the blood, 697, 704–709
  in the intestine, 403
  in the lymph, 251, 702
  in the stomach, 372
  in the swimming-bladder of fishes, 710
  in secretions, 702, 703
  in transudates, 703
  , tension of, in blood, 203, 698
  , lack of, action on protein destruction, 461, 569, 611
  , lack of, action on elimination of lactic acid. 461, 469, 608
  , lack of, action on elimination of sugar, 461
Oxygen capacity, specific, 711, 727
Oxygen-carriers, 7, 648, 649
Oxygen, calorific value in the combustion of different foods, 623-624, 723
Oxygen, consumption in the blood,
Oxygenases, 17
Oxyhæmatin, 210
Oxyhæmocyanin, 219
Oxyhæmoglobin, 198
  dissociation of, 198–200, 704, 705
  , properties and reactions, 198–202
  , quantity of, in the blood, 196, 197, 239, 241–246

Oxyhæmoglobin, quantity in the muscles, 454
, passage of, into the urine, 648
, behavior with gastric juice, 360
, behavior with trypsin, 395
Oxyhydroparacoumaric acid, 597
Oxymandelic acid, 597, 600
Oxymonamino acids, 29, 33, 95
Oxymonaminosuberic acid, 29, 96
Oxymonaminosuccinic acid, 29, 96
Oxynaphthalene, 632
Oxyphenyl-acetic acid, 90, 401, 596, 597, 637
Oxyphenylaminopropionic acid. See Tyrosine.
Oxyphenylethylamine, 30, 54
Oxyphenylpropionic acid, 401, 597, 637
Oxyproteic acid in urine, 612, 613
Oxyproteins, 31
Oxyprotosulphonic acid, 31
Oxypyrrolidincarboxylic acid, 34, 102
Oxyquinoline, 638
Oxyquinolinecarboxylic acid, 600
Ozone, 3
Ozone transmitter, 201

Palmitic acid, 134
Palmitin, 134
Pancreas, 381
, relation to glycolysis, 185, 302, 396
, extirpation of, action on absorption,
, extirpation of, elimination of sugar, 418, 421, 425
, pepsin, 385
, change during secretion, 381
Pancreatic calculi, 396
Pancreatic diabetes, 301, 302
Pancreatic diastase, 388
Pancreatic protein, 150, 381
Pancreatic rennin, 396
Pancreatic casein, 396
Pancreatic juice, 382–388
, secretion of, 382–385
, enzymes of, 386
, action on foodstuffs, 387–395
, action upon peptides, 35, 396
Parabamic acid, 568
Paracasein, 522
Parachymosin, 361, 362
Paracresol, formation in putrefaction, 401, 588
Paraglobulin. See Serglobulin.
Paraglycocholic acid, 313
Parahæmoglobin, 201
Parahistone, 63
Paralactic acid, 460
, relation to formation of uric acid, 572, 573
Paralactic acid, properties and occurrence, 460–463
, formation from glycogen, 461, 466, 467
, formation in osteomalacia bones, 439
, formation in muscle during work, 468–470, 472, 473
, formation in rigor mortis, 467
, formation in lack of oxygen, 460, 461, 469
, formation in animals with extirpated livers, 460, 461, 572
, passage of, into the urine, 460–462, 572, 608
Paralbumin, 275, 500
Paralytic saliva, 340
Paraminophenol, 633
Paramucin, 500
Paramyosinogen, 450, 452
Paranuclein. See Pseudonuclein.
Paranucleic acid, 522
Paraoxyphenylacetic acid, 90, 401, 596, 597, 637
Paraoxyphenylaminopropionic acid, 89, 401, 596, 597, 637
Paraoxypropiophenone, behavior in animal body, 638
Parapeptone, 359
Paraxanthine, 157, 580
in urine, 578, 580
Parenterally introduced protein, 412
Parietal or delomorphic cells, 349, 364
Parotid, 339
Parotid saliva, 341
Parovarial cysts, 501
Partition of the nitrogen in the urine, 548, 549, 568, 569, 611
Peas, utilization in the intestine, 421
Pemphigus chronicus, 265
Penicillium glaucum, 86
Pennacerin, 692
Pentacrinin, 691
Pentamethylendiamine. See Cadaverin.
Pentosanes, 110
digestion of, 427
Pentoses, 110
, relation to glycogen formation, 111, 290
in blood, 184
in urine, 110, 666
in pancreas, 110
in nucleic acids, 152, 155
in nucleoproteids, 72, 110, 285, 514
Penzoldt, acetone reaction, 671
Pepsin, 354–361
, detection in gastric contents, 373
, quantitative estimation, 357
, occurrence in the urine, 426, 615
Pepsin charge, in the stomach, 364
, in the pancreas, 385

Pepsin cells, 349
Pepsin digestion, 356–362
, products of, 52, 53, 359
Pepsin glands, 348
Pepsin-glutin peptone, 57
Pepsin-hydrochloric acid, 360, 361
Pepsin-like enzyme, 354
Pepsinogen, 364
Pepsin peptones, 51, 57
Pepsin test, 356
Peptides, 35, 54, 394, 395, 416, 614
, relation to trypsin, 35, 395
Peptochondrin, 433
Peptones, 29, 30, 36, 49–61, 359
, assimilation of, 413–417
, absorption of, 414, 415
, passage of into urine, 414, 643
Peptone blood, 235
Peptone-plasma, 171
Peptozym, 234
Percaglobulin, 510
Perch eggs, 66, 504, 509, 510
Pericardial fluid, 257, 260
Perilymph, 494
Peritoneal fluid, 257, 261
Permeability, of the blood-corpuscles, 195, 196
of the vascular walls, 257, 258
of the muscles, 465
Peroxidases, 17, 18
. See also the tissues and fluids.
Peroxyproteic acid, 31
Perspiratio insensibilis, 717
Perspiration, 692–695
Pettenkofer's test for bile-acids, 135, 312, 652
respiration apparatus, 712,
Phacozymase, 493
Phaseomannite, 458
Phenaceturic acid, 588, 634, 635
Phenol-glucuronic acid, 590, 610, 638
Phenol-sulphuric acid in the urine, 589, 592, 637
in sweat, 594
Phenols, elimination by the urine, 401, 588–591, 633, 637, 638
in starvation, 404
, estimation in urine, 590, 591
, formation in putrefaction, 30, 401, 588
, behavior in the animal body, 401, 402, 588, 589, 637, 638
Phenylacetic acid, formation in putrefaction, 30, 401
, behavior in the animal body, 588, 634, 635
Phenylalanine, 33, 91
, behavior in the animal body, 585, 633
, in alcaptonuria, 597–599
Phenylaminoacetic acid, behavior in the animal body, 634
Phenylaminopropionic acid, 91
Phenylbutyric acid, 634
Phenylglucosazone, 107, 117
Phenylhydrazine test, 107, 117
in the urine, 657
Phenylketopropionic acid, 633, 634
Phenyllactic acid, 598, 633, 635
Phenyllactosazone, 524
Phenylpropionic acid, behavior in the animal body, 585, 634
Phenylpropionic acid, formation in putrefaction, 30, 401, 585
Phenylvalerianic acid, 634
Phlebin, 196
Phlorhizin, poisoning with, 282, 297, 610, 670
Phlorhizin diabetes, 297, 610
Phloroglucin as reagent, 111, 374, 666
Phosphate calculi, 681
Phosphates in urine, 543, 619–622, 640, 677–682
. See also the different phosphates, 143, 144
Phosphatides, 146, 325, 328, 480, 481, 488
Phosphaturia, 620
Phosphocarnic acid, 454, 457
in the milk, 523, 532
in blood, 186
in brain, 480, 481
in the urine, 614
in relation to the elimination of CO_2 and lactic acid, 462
in relation to muscular activity, 470, 474
Phosphoglucoproteid, 71, 509
Phosphoric acid, elimination by the urine, 617–622, 625, 628
, formation in muscular activity, 470
, quantitative estimation of, 620–622
Phosphorized combinations in the urine, 614
Phosphorus poisoning, action on the elimination of ammonia, 554
, action on the elimination of urea, 548, 554
, action on the elimination of lactic acid, 460, 462, 608
, action upon the blood, 172, 175
, fatty degeneration caused by, 282, 283, 443
, liver autolysis in, 23, 283, 284, 285

Phosphorus poisoning, change in the urine. 285, 460, 548 554, 613
Photomethæmoglobin, 205
Phrenin, 486
Phrenosin, 483, 485, 488
Phthalic acid, behavior in the body, 633
  from cholic acid, 315
Phthalimide malonic ester, 102
Phyllocyanin, 214
Phylloporphyrin, 197, 214
Phylloerythrin, 324
Phymatorusin, 688
  in the urine, 651
Physetoleic acid, 138
Physiological availability, 727
Phytosterines, 335
α-Picoline, behavior in the animal body, 637
Picric acid, reagent for protein, 42, 646
  , reagent for creatinine, 565 567
  , reagent for sugar, 117, 566
Pigment calculi, 334
Pigments of the eye, 489–491
  of the blood, 196–219
  of the blood-serum, 186, 187, 505
  of the corpora lutea, 216, 498
  of the egg-shell, 509
  of feathers, 690
  of the fat-cells, 441
  of the bile, 319–325, 328, 331
  of the urine, 600-607
  of the skin, 688-691
  of the lobster, 509, 690
  of the liver, 282
  of the muscles, 453, 454
  of lower animals, 218, 219
  , medicinal pigments in the urine, 639, 654
Pigmentary acholia, 329
Pig's milk, 529
Pike, flesh of, 476
Pilocarpine, action on the secretion of intestinal juice, 377
  , action on the elimination of CO_2 in the stomach, 372
  , action on the secretion of saliva, 347
  , action on the elimination of uric acid, 569
Piqûre, 299
Piria's tyrosine test, 90
Placenta, 512
Plant gums, 128, 129
Plant nucleic acids, 156
Plants, chemical processes in, 1, 2
Plasma. See Blood-plasma.
Plasminic acid, 156
Plasmoschisis, 227
Plasmozym, 232
Plastein, 56, 363, 396
Plasteinogen, 57
Plastin, 149
Plattner's crystallized bile, 311
Plethora polycythæmia, 245
Pleural fluid, 257, 261
Plums, action on the elimination of hippuric acid, 585
Pneumonic infiltration, solution of, 23, 268, 713
Poikilocytosis, 247
Polarization test, 664
Polycythæmia, 245, 248
Polypeptides. See Peptides.
Polyperythrin, 691
Polysaccharides, 126
Polyuria, 628
Pons varolii, 486
Poorhouses, diet of inmates of, 770
Pork, 475
Pork-fat, absorption of, 423
Portal vein, blood of, 241, 295, 296, 419
Positive phase, 234
Potassium combinations, division of, in the form-elements and fluids, 166, 167, 464
  , elimination of, in fevers, 623
  , elimination of in starvation, 623, 731
  , elimination by the saliva, 347
  in the urine, 623
Potassium chlorate, poisoning with, 203
Potassium phosphate in yolk of eggs, 505
  in muscles, 464, 465, 477
  in cells, 166, 167, 168
  in spermatozoa, 496
Potassium sulphocyanide in the urine, 611
  in saliva, 341, 343
  in gastric contents, 354
Potatoes, absorption of, in the intestine, 421
Potential energy of various foods, 724–728
Precipitins, 186, 413
Preglobulin, 141, 228, 271
Preputial secretion, 692
Primary proteoses, 52
Prisoners, food-ration for, 770
Proliferous cysts, 498
α-Proline, 34, 74, 101
Prolineglycyl anhydride, 35
Propepsin, 364
Propeptones, 50
Propylalanine, 35
Propyl benzene, behavior in the animal body, 634
Propylene glycol, relation to formation of glycogen, 291
Prosecretin, 379, 384
Prostatic calculi, 498

Prostatic secretion, 496
Prosthethic group, 71
Protagon, 148, 271, 480, 481, 482
Protalbinic acid, 49
Protoproteoses, 51
Protamines, 36, 58, 62, 63, 149, 497, 498
Proteids, 36, 37–65.
  . See also the various protein groups.
Protein, separation from fluids, 44
  , approximate estimation in the urine, 646
  , circulating and tissue protein, 741–745
  , action on the formation of glycogen, 292, 294, 303–305
  , active, 4
  , living and non-living, 4
  , detection and quantitative estimation of, 43–45, 639–646
  , regeneration of, 415, 416, 417
  , absorption of, 412–418
  , passage of, into the urine, 640
  , heat of combustion of, 723–726
  , digestibility in gastric juice, 356, 368
  , digestibility in pancreatic juice, 393
  , formation of sugar from, 303–305
Protein bodies in general, 26–83
  , summary of the various, 36, 37
  . See also the various protein bodies of the tissues and fluids.
Protein hydrogele, 39, 40
Protein hydrosole, 39, 40
Protein content affected by inoculation, 188
Protein metabolism in work and rest, 471–475, 758
  in starvation, 728, 729
  in various ages, 757, 758
  with different foods, 739–745
  ante-mortem increase, 730
  after feeding with thyroid extracts, 276
Protein overfeeding, 751, 752
Protein putrefaction, 30, 401–408, 585, 588, 589
Protein, relation to the albuminates, 49
Proteincystine, 92
Proteinochromogen, 30, 102
Protein substances, 26–82
  , synthesis of, 34, 35
  , action upon coagulation of the blood, 233
  . See also individual protein bodies.
Proteoses, 36, 52
  , general properties and preparation, 50–61
  in blood, 183, 247, 415
  , formation in protein putrefaction, 50, 401, 415, 416
  , relationship to the coagulation of the blood, 171, 226, 234, 235
  , nutritive value, 745, 746
  , absorption of, 414–417
  , transformation into protein, 415
  , occurrence in urine, 643
Prothrombin, 176, 231, 232, 233
Protic acid, 454
Proteolytic enzymes, 16, 263
Protocatechuic acid, behavior in the body, 591
Protoelastose, 76
Protogelatose, 79
Protogen, 49
Protokyrin, 58
Protones, 63
Protoplasm, 4, 140, 141
  and protein decomposition of, 548
Protosyntonose, 100
Pseudocerebrin, 485
Pseudochylous fluid, 262
Pseudoglobulin, 179, 643
Pseudoglycogen formers,
Pseudohæmoglobin, 203 293
Pseudolevulose, 108
Pseudomucin, 68, 500
  in ascitic fluids, 262
  in cysts, 500
  in the gall-bladder, 329
Pseudonucleins, 47, 151, 359,
  from casein, 521, 531
  from vitellin
Pseudopepsin, 354
Pseudotagatose, 108
Pseudoxanthine, 457
Psittacofulvin, 690
Psylla-alcohol, 692
Psyllic acid, 692
Psychical period of secretion, 350
Ptomaines, 24
  in the urine, 615, 676
Ptyalin, 343, 344
  . behavior with acid, 345, 366
  . action on starch, 344–346
  , tests, 345, 346
Pulmotartaric acid, 713
Purine, 157
Purine bases, 156, 578
  , See also Nuclein bases.
Purple, 691
Purple cruorin, 202
Pus, 266–269
  , blue, 269
  cells, 267
  in urine, 651
  corpuscles, 267
  serum, 266

Putrefactive processes, 25, 30
in intestine, 400–408, 585, 588–596
Putrescine, 24, 97
in intestine, 24, 676
in the urine, 615, 676
Pyin, 261, 266, 269
Pyinic acid, 269
Pyloric gland, 348
Pyloric secretion, 365
Pyocyanin, 269
in sweat, 695
Pyogenin, 268, 483
Pyosin, 268, 483
Pyoxanthose, 269
Pyridine, behavior in the body, 639
α-Pyridine-carboxylic acid, 632, 636
Pyrocatechin, 591
, occurrence in urine, 591
, occurrence in transudates, 259, 265
Pyrocatechin-sulphuric acid, 591
Pyromucic acid, 637
Pyromucinornithuric acid, 637
Pyrrol derivatives, 210, 689
α-Pyrrolidinecarboxylic acid, 101–103, 197. See α-Proline.
Pyrrolidonecarboxylic acid, 74

Quercite, relation to glycogen formation, 291
Quinic acid, behavior in the animal body, 586
Quinine, passage of, into urine, 639
, passage of, into sweat, 695
, action of, on the elimination of uric acid, 569
, action on the spleen, 274
Quotient, respiratory, 306, 446, 472, 722 731, 760
Quotient, urea to nitrogen, 628, 720
, nitrogen to sugar, 304, 306

Racemic acid, behavior in the animal body, 539
Rachitis, bones in, 438, 440
Rape-seed oil, feeding with, 442
Reductases, 19
Reduction processes, 2, 5, 18, 19. See also the various chapters.
Reichert-Meissl's equivalent, 137
Reindeer, milk of, 529
Rennin, 25, 56. See also Chymosin.
Rennin cells, 349
Rennin glands, 349
Rennin zymogen, 349, 361, 362
Reproductive organs, 419–513
Resacetophenon, 637
Residual nitrogen, 183
Resin acids, transition into urine, 639, 641
Respiration, anaerobic, 21, 461
, external, 696, 703
, internal, 696, 703, 712
of the hen's egg, 511, 512
Respiration of plants, 2
. See also Chemistry of respiration, 696–714, and Exchange of gas under various conditions.
Respiratory quotient; 306, 446, 472, 722, 731, 760
Rest, metabolism during, 467–472, 758–761
Reticulin, 37, 80, 428
Retene, 335, 336
Retina, 489
Reversion, 124
Revertose, 16
Reynolds' acetone reaction, 671
Rhamnose, relation to glycogen formation, 290, 336
Rheometer, 706
Rhodizonic acid, 458
Rhodophan, 491
Rhodopsin, 489
Rhubarb, action on the urine, 639
Rib-cartilage, 434
Rigor mortis of the muscles, 465
Roberts' method of estimating sugar, 663
Roch's reaction for protein, 642
Rodents, bile-acids of, 318
Rods of the retina, pigments of, 490
Rosenbach's bile-pigment test, 852
urine test, 675
Rotation, specific, 109
Rosin's levulose reaction, 119, 665
Rovida's hyaline substance, 141, 221, 267
Rubner's sugar test, 117, 665
Rye bread, utilization, 417, 421, 727

Saccharic acid, 106, 107, 121
, lactone of, 121
, relation to glycogen formation, 291
, behavior in diabetes, 300
Saccharose, 123, 124
calorific value, 724
absorption of, 419
Salicylase or aldehydase, 18
Salicylic acid, action on pepsin digestion, 359
, action on trypsin digestion, 393
, behavior in the animal body, 635
Salicylic-acid amyl ester, 284
Salicylsulphonic acid as protein reagent, 42
Saliva, 339–348
, secretion of, 346, 347
, mixe4, 342
, physiological importance, 348
, behavior in the stomach, 348, 366, 367
, action of, 345, 348, 367
, gases of, 340, 702
, composition of, 346, 347
Salivary calculi, 348
Salivary diastase. See Ptyalin.

Salivary glands, 339
Salkowski's cholesterin reaction, 336
Salkowski-Ludwig's method of estimating uric acid, 336
Salmine, 63, 64
Salmon, flesh of, 454
  , sperma of, 63, 155, 497
Salmonucleic acid, 154
Salts, action of, upon metabolism, 754
  , antagonistic action of, 377, 375
  . See also the various salts.
Salt-plasma, 171
Salts of vegetable acids, behavior in the organism, 544
Samandarin, 692
Santonine, action on the urine, 639, 654
Sapokrinin, 385
Saponification equivalent, 136
Saponification, 133, 388, 398, 422, 423
Saponin, 193, 337
Sarcolactic acid. See Paralactic acid.
Sarcolemma, 447
Sarcomelanin, 688
Sarcomelanic acid, 688
Sarcosine, 455
  , behavior in the animal body, 630
Sarkine. See Hypoxanthine.
Scherer's inosite test, 459
Schiff's reaction for cholesterin, 336
  reaction for uric acid, 576
  reaction for urea, 555
Schreiner's base, 496
Schütz-Borissow's law, 390, 392
Schweitzer's reagent, 107
Sclerotic, 493
Scombrine, 59, 63, 64
Scombron, 62
Scyllite, 272
Scymnol, 310
Scymnol-sulphuric acid, 310
Seal-fat, 138
Sea-urchin, sperm of, 62
Sebacic acid, 135
Sebum, 691
Secondary proteoses, 52
Secretin, 309, 377, 379, 384
Secretin enzymes, 22
Sediments. See Urinary sediments.
Sedimentum lateritium, 543, 575, 607, 677
Seliwanoff's reaction for levulose, 119, 665
Semen, 495–497
Semicarbazide, poisoning with, 584
Semiglutin, 79
Seminose. See Mannose.
Senna, action on the urine, 639, 654
Sepsine, 24
Seralbumin, 36, 181
  , detection of, in the urine, 640, 643
  , quantitative estimation of, 183, 645
  , absorption of, 413
Serglobulin, 36, 178
  , detection of, in the urine, 640, 643
  , quantitative estimation of, 180, 645
Sericin, 37, 82
Serine, 33, 95
Serolin, 183
Serosamucin, 258
Serous fluids, 256–265
Serum. See Blood-serum.
Serum casein. See Serglobulin.
Sex, influence on metabolism, 756
Sharks, bile of, 310, 325
  , urea in, 240, 325, 547
Sheep's milk, 529
Shell-membrane of the hen's egg, 73, 509
Silicic acid in feathers, 685
  in hair, 685
  in urine, 626
  in hen's egg, 505, 509, 510
  in connective tissue, 429
Silicic acid ester in feathers, 685
Silk gelatine, 82
Sinistrin, animal, 71
Silver, in blood, 239
Skatol, 29, 34, 102, 401, 402
  , formation in putrefaction, 29, 401, 588
  , behavior in the animal body, 400, 401, 588, 595, 634
Skatolacetic acid, 30, 102
Skatolaminoacetic acid, 30, 102
Skatolcarboxylic acid, 102, 596
Skatol-pigment, 595, 596, 607
Skatosine, 103
Skatoxyl, 401, 588, 634
Skatoxylglucuronic acid, 595
Skatoxylsulphuric acid, 588, 595
  in sweat, 694
Skeletins, 81
Skeleton at various ages, 438
Skin, 685–695
  , excretion through, 690, 692–695, 717
Sleep, metabolism, 761
Small intestine, 378, 380
  extirpation of, 427
Smegma præputii, 692
Smith's reaction for bile-pigments, 654
Smooth muscles, 477
Snail mucin, 66
Snake poison, action upon blood, 193, 226, 234
Soaps in blood-serum, 183
  in chyle, 251, 423
  in pus, 268
  in excrements, 408, 426
  in bile, 310, 325
  in milk, 532
  , importance of, in the emulsification of fats, 389, 390, 398, 423
Sodium alcoholate as a saponification agent, 136, 659

Sodium chloride, elimination by the urine, 616, 617, 694, 695
  , elimination by the sweat, 694, 695
  , physiological importance, 736
  , quantitative estimation, 616–619
  , influence on the quantity of urine, 754
  , influence on the elimination of urea, 754
  , influence on the secretion of gastric juice, 364, 736
  , behavior with food rich in potassium, 736
  , insufficient supply of, 364, 736
  , action on the secretion of intestinal juice, 377, 378
  , action on pepsin digestion, 358, 359
  , action on trypsin digestion, 393, 394
Sodium compounds, elimination by the urine, 623
  , division among the form-elements and fluids, 166
  . See also the various tissues and fluids.
Sodium phosphate in the urine, 619, 620
Sodium salicylate, action on the secretion of bile, 309
Sodium tartrate, relation to glycogen formation, 291
Solanin, 193
Soldiers, diet of, 769
Sorbin, 119
Sorbinose, 113, 119
Sorbite, 106
Source of muscular energy, 472–474
Spawn of the frog, 71
Specific rotation, 109
Spectrophotometry, 218
Sperma, 63, 495–498
Spermaceti, 138
Spermaceti oil, 138
Spermatin, 498
Spermatocele fluids, 263
Spermatozoa, 497
Spermine, 496
Spermine crystals, 496
Spherules, 37, 503, 509
Sphingosin, 485
Sphygmogenin, 278
Spider excrement, guanin therein, 160
Spiegler's reagent, 642
Spirographin, 69
Spirogyra, 114, 167
Spleen, 272–275
  , relation to formation of blood, 274
  , relation to formation of uric acid, 274, 570, 573
  , relation to digestion, 385
  , blood of the, 242
Spleen pulp, 272
Splitting processes in general, 1, 2, 9. See also the various enzymes.
Spongin, 37, 81, 82
Sputum, 714
Sputum mucin. See Mucin from mucous membrane, 66
Starch, 126
  , hydrolytic cleavage by diastase, 128, 387, 388
  , hydrolytic cleavage by pancreatic diastase, 388
  , hydrolytic cleavage by saliva, 344
  , calorific value, 724
  , absorption, 419, 421
Starches, digestion of, 367, 388
Starch, cellulose, 126
Starch granulose, 126
Starvation, action on the blood, 244, 731, 732
  , action on the urine, 404, 548, 585, 592
  , action on the elimination of indican, 404, 592
  , action on the elimination of oxalic acid, 582
  , action on the secretion of bile, 307, 308
  , action on the secretion of pancreatic juice, 382
  , action on the elimination of phenol, 404
  , action on metabolism, 722, 728–733
  , death from, 728
Starvation cures, 770, 771
Starvation requirement, 733, 755
Steapsin, 388
Stearic acid, 133
Stearin, 133
  , absorption of, 423
Stentorin, blue, 691
Stercobilin, 320, 409, 603
Stercorin, 337
Stethal, 138
Stokes's reduction fluid, 203
Stokvis' reaction for bile-pigments, 653
Stomach, gases in the, 372
  , importance in digestion, 369
  , pepsin charge in, 364
  , relation to intestinal putrefaction, 371, 406, 407
  , auto-digestion of, 372
  , digestion in the, 365–372
Stomachic glands, 350
Stone-cystine, 92
Streptococcus, behavior with gastric juice, 371

Stroma fibrin, 195
Stroma of the blood-corpuscles, 194
  of the muscles, 451
  of the ovaries, 498
Strontium salts and blood coagulation, 171
Struma cystica, 275
Strychnine, passage of, into the urine, 639
  and sugar elimination, 299
Sturgeon, sperma of, 63
Sturine, 63, 100
Sublingual glands, 339
Sublingual saliva, 341
Submaxillary glands, 339
Submaxillary mucin, 66, 67
Submaxillary saliva, 340, 341
Succinic acid in putrefaction, 32
  in the fermentation of milk, 516
  in the intestine, 400
  in the spleen, 272
  in transudates, 259, 264
  in the thyroid glands, 275
  from phosphocarnic acid, 457
  , passage of, into the urine, 630
  , passage of, into the sweat, 695
Sugar, relation to work, 469, 473
  , formation from fats, 306, 473
  , formation from protein, 303–305
Sugar formation, in the liver, 295–301, 306
  after pancreas extirpation, 301–305
Sugar, behavior on subcutaneous injection, 293
  , behavior to blood-corpuscles, 195
  , quantitative determination, 659–665
  . See also various kinds of sugar.
Sugar tests in the urine, 655–659
Sulphæmoglobin, 207
Sulphocyanides in the urine, 611, 631
  in gastric juice, 354
  in the saliva, 341, 343
Sulphonal intoxication, urine in, 213, 650
Sulphonic acids, behavior in the animal body, 540
Sulphur, of proteins, 27. See also various proteins.
  , in the urine, 471, 611, 612
  , elimination of, in activity, 471
  , elimination of, with lack of oxygen, 611
  , neutral and acid sulphur in urine, 611
  , behavior in the organism, 611, 631
Sulphur methæmoglobin, 207
Sulphuretted hydrogen in putrefaction in the intestine, 401, 404
  in the urine, 612
Sulphuric acid, ethereal and sulphate, in the urine, 588, 589, 611, 622, 623
  , elimination of, in activity, 471
  , elimination of, by the urine, 543, 622, 623, 628
  , elimination of, by the sweat, 694
  , estimation of, 622
  , relation to elimination of nitrogen, 471, 611, 622
  , action on pepsin digestion, 358
Suprarenal capsule, 277
Suprarenin, 278. See also Adrenalin.
Swallow's-nests, edible, 69
Sweat, 692–695
Swimming-bladders of fishes, gases of, 710
  , guanine in, 160
Sympathetic saliva, 340
Synproteose, 55
Synovia, 265
Synovial fluid, 265
Synovial mucin, 258
Synoviamucin, 265
Synovin, 266
Synthesis, 1, 2,
  of ethereal sulphuric acids, 280, 401, 588, 590, 593, 594, 637
  of conjugated glucuronic acids, 122, 589, 593, 609, 610, 632, 638
  of uric acid, 567, 568, 572, 573
  of urea, 547, 550, 551, 552
  of hippuric acid, 3, 585, 635
  of varieties of sugars, 106, 114
  of polypeptides, 36,
Syntonin, 48, 100
  , calorific value of, 726

Tagatose, 108
Talonic acid, 120
Talose, 108, 113, 120
Tapeworm cysts, 265
Tannic acid, behavior in the animal body, 637
Tartar, 348
Tartaric acid, relation to glycogen formation, 291
  , passage of, into sweat, 695
  , behavior in the animal body, 630
Tartronic acid, 573
Tatalbumin, 506
Taurine, 94, 95, 310, 313, 331
  , behavior in the animal body, 629
Taurocarbamic acid, 631
Taurocholeic acid, 314
Taurocholic acid, 310, 313, 328
  , occurrence in meconium, 410
  , decomposition in the intestine, 404

Taurocholic acid, protein-precipitating action, 42, 647
Tea, action on metabolism, 755
Tears, 418
Teeth, 440
Teichmann's crystals, 211, 649
Tendon mucin, 66
Tendon mucoid, 428
Tendon synovia, 265
Tension of the CO_2 in the blood, 708–710
    in the tissues, 712
    in the lymph, 251
    O in the blood, 703–708
Terpen-glucuronic acid, 667
Terpentine, action of, on the secretion of bile, 308
    , action of, on the urine, 639
    , behavior in the animal body, 609, 638
Tetraglycylglycine, 35, 396
Tetraoxyaminocaproic acid, 96
Tetrapeptides, 35, 395
Tetronerythrin, 219, 690
Testes, 495
Tetroses, 105
Theobromine, 157
    , behavior in the animal body, 579
Theophylline, 157
    , behavior in the animal body, 579
Thioalcohols, behavior in the animal body, 632
Thioglycolic acid, 74
    , behavior in the animal body, 632
Thiolactic acid, 28, 33, 94
Thiophene, behavior in the animal body, 637
Thiophenic acid, 637
Thiophenuric acid, 637
Thiotolene, 637
Thrombin, 13, 16, 175, 228, 233
Thrombogen, 231–233
Thrombokinase, 231
Thrombosin, 229
Thymine, 152, 154, 165
Thymic acid, 154
Thymonucleic acid, 151, 152, 153, 154, 155
Thymus, 270
Thyreoglobulin, 276, 277
Thyreoidea, 275–277
Thyreoproteid, 276
Thyreotoxalbumin, 277
Thyroid gland, 275, 276
Thyroiodin. See Iodothyrin.
Tissue-fibrinogen, 141, 271
Tissue proteids.
Tollens' reaction for pentoses, 111, 112
Toluene, behavior in the animal body, 585, 634
Toluric acid, 635
Toluylenediamine, poisoning with, 333
Toluic acid, 635
Tonus, chemical of the muscle, 467
Tooth structure, 440
Tortoise, bones of, 436
Tortoise-shell, 73, 691
Toxalbumins, behavior with gastric juice, 371
Toxines, 24, 25, 186, 280
Tracheal cartilage, 420, 433
Transudates, 256–266
Tribromacetic acid.
Tricalcium casein, 520
Trichloracetic acid as reagent, 42, 45
Trichlorethyl-glucuronic acid. See Urochloralic acid.
Triglycylglycine, 35, 396
Triolein, 34
Trioses, 105
Tripalmitin, 134
Tripeptides, 35, 395
Triple phosphate in urinary sediments, 678, 679
    in urinary calculi, 680, 681, 682
Tristearin, 133
Triticonucleic acid, 152, 156
Trommer's test for sugar, 116, 655
    , behavior with uric acid, 575
    , behavior with creatinine, 565
Tropics, metabolism in inhabitants of, 762
Trypsin, 186, 390–395
    , action on proteins, 54, 55, 392, 416
    , action on peptides, 35, 395, 396
    , importance in absorption, 416
Trypsin digestion, 392–396
    , products of, 394
Trypsinogen, 383–386
Trypsin peptone, 51, 54, 55, 57, 58
Tryptophane, 30, 102, 355
Tubo-ovarial cysts, 501
Tunicin, 685
Turacin, 690
Turacoverdin, 690
Tryosine, 18, 33, 54, 89–91
    , in urine, 675
    , in sediments, 680
    , detection of, 91, 675
    , behavior in putrefaction, 400, 585, 588
    , behavior in the animal body, 598, 599, 633, 670
Tryosinases, 18, 90, 690
Tyrosine-sulphuric acid, 90

Uffelmann's reaction for lactic acid, 374
Umikoff's reaction, 532, 533
Uracil, 152, 156, 164
Uræmia, bile in, 329
    , gastric contents in, 373
    , sweat in, 694
Uraminobenzoic acid, 636
Urates, 542
    , in sediments, 575, 678

Urea, 547
    , elimination in starvation, 548
    , elimination in children, 549, 665
    , elimination in disease, 547, 548, 554
    , properties and reactions, 554
    , formation and origin, 550–554, 630
    , quantitative estimation, 558–562
    , synthesis, 547, 551–553
    , occurrence in the blood, 186, 240, 242, 551
    , occurrence in the bile, 325, 329, 547
    , occurrence in the liver, 547, 550, 551
    , occurrence in the muscles, 455, 547
    , occurrence in transudates, 259
Urea glucuronic acid, 609
Urea nitrate, 555
Urea oxalate, 555
Ureides, 30, 568, 583
Urein, 562
Urethane, 563
Urease, 16, 677
Ureido-glucuronic acid, 609, 611
Uric acid, 157, 550, 567, 568
    , elimination in disease, 570
    , elimination after feeding with nuclein, 569, 571
    , relation to urea, 567–573
    , properties and reactions, 573–576
    , formation in the animal body, 570–574
    , quantitative estimation, 576–578
    , syntheses of, 568, 572, 573
    , behavior in the animal body, 573, 574
    , occurrence of, 568, 569
    , occurrence of, in sweat, 547, 694
    , occurrence of, in sediments, 543, 575
Uric-acid calculi, 681
Urinary calculi, 680–683
Urinary pigments, 601–607
    , medicinal, 639, 654
Urinary sand, 680
Urinary sediments, 542, 543, 677–680
Urine, 541–684
    , excretion of, 626, 627
    , inorganic constituents of, 616–626
    , poisonous constituents of, 615
    , organic pathological constituents of, 639–676
    , physiological constituents of, 546–616
    , enzymes of, 615
    , casual constituents of, 629–639
    , color of, 542, 601, 628, 639, 648–654
    , solids, calculation of, 626, 627
    , quantity of solids, 626, 627
    , alkaline fermentation of, 677
    , acid fermentation of, 677
    , gases of, 626, 703, 712
    , quantity of, 626, 627
    , physical properties of, 542–547
Urine, osmotic pressure of, 546
    , physico-chemical analysis of, 628
    , reaction of, 543–546
    , acidity of, 543–545
    , estimation of acidity, 545
    , specific gravity of, 546, 627, 628
    , passage of foreign bodies into, 629–639
    , composition of, 628
    , reducing power of, 608
Urine indican, 592
Urine indigo, 592, 601
Urine poison, 615
Urine purines, endogenous and exogenous, 578–581
Urine sugar. See Dextrose.
Urinometer, 546
Urobilin, 601, 602–607
    , relation to bilirubin, 319, 332, 404, 603
    , relation to choletelin, 603
    , relation to hæmatin, 332, 603
    , relation to hæmatoporphyrin, 214, 603
    , relation to hydrobilirubin, 332, 603
Urobilin icterus, 604
Urobilinogen, 601, 605
Urobilinoid bodies, 603
Urocarnic acid, 526, 615
Urochloralic acid, 122, 632
Urochrome, 601, 602
Urocyanin, 601
Uroerythrin, 601, 607
Uroferric acid, 611, 613
Urofuscohæmatin, 650, 651
Uroglaucin, 601
Urohæmatin, 601
Urohodin, 601
Uroleucic acid, 597, 60
Uromelanins, 601
Uronitrotoluolic acid, 638
Urophæin, 601
Uroproteic acid, 613
Urorubin, 601
Urorubrohæmatin, 650
Urorosein, 596, 601, 651
Urospectrin, 650
Urostealith calculi, 682
Urotheobromine, 580
Urotoxic coefficient, 615
Uroxanthine, 592
Uroxonic acid, 568
Ursocholeic acid, 319
Uterine milk, 512
Uterus colloid, 502
Utilization of the various foodstuffs, 417, 421, 425, 530, 531

Valerianic acid, 29
Vegetable acids, behavior of the alkali salts of, in body, 544
Vegetable gums, 120, 129
Vegetable mucilages, 128, 129

Vegetarians, food of, 750, 767
  , excrement, 408
Vernix caseosa, 336, 691
Vesicatory blisters, 265
Vesicle calculi,
Vesiculase, 496
Virtual sugar, 184
Visual purple, 489–491
Visual red, 489
Vitali's pus-blood test, 649
Vitellin, 36
  in yolk of egg, 503
  in protoplasm, 141
Vitellolutein, 505
Vitellorubin, 505
Vitelloses, 52
Vitreous humor, 491

Water, drinking of, action in the elimination of chlorides, 616
  , action on the elimination of uric acid, 569
  , action on the elimination of urea, 753
  , action on the deposition of fat, 754
  , action on the excretion of urine, 626
  , elimination of, through the urine, 626–628, 717
  , elimination of, through the skin, 693, 717
  , elimination of, in starvation, 731
  , elimination of, importance for the animal body, 734
  , elimination of, quantity of, in the various organs, 734
  , elimination, lack of, in the food, 734
Wax, 138
  in plants, 691
Weidel's xanthine reaction, 160
Weyl's reaction for creatinine, 565
Wheat bread, absorption of, 418
Whey, 516, 528
Whey proteid, 521
White of egg, 506–510
  , calorific value of, 724
  , absorption of, 413, 414
Witch's milk, 534
Woman's milk. See Human milk.
Wool-fat, 337, 692
Work, action on the elimination of chlorine, 616
  , action on the elimination of sulphur, 471
  , action on the excretion of nitrogen, 470, 471
  , action of the necessity for food, 768, 769
  , action on metabolism, 468–474, 758–761
Worm-Müller's sugar test, 655
Wound secretion, 265

Xanthine, 157, 159
  in the urine, 578
  in urinary calculi, 682
  in urinary sediments, 680
  , detection and quantitative estimation, 159, 160, 163, 164, 580, 581
Xanthine bodies. See Nuclein bases.
Xanthine calculi, 682
Xanthine oxidase, 18, 73, 571
Xantho-creatinine, 457, 470, 567
Xantho-melanin, 32
Xanthophan, 491
Xanthophyll groups, 505
Xanthoproteic reaction, 42
Xylene, behavior in the animal body, 634
Xyliton, 689
Xyloses, 113, 282
  , relation to the formation of glycogen, 111, 290

Yeast-cells, relation to fermentation, 10, 11
Yeast maltase, 17
Yeast nucleic acid, 152, 156
Yeast nuclein, 150
Yolk of the hen's egg, 502–506
Yolk-spherules, 37, 503, 509

Zein, 27, 98
Zinc in the bile, 325
  in the liver, 286
  , passage of, into milk, 539
Zooerythrin, 690
Zoofulvin, 690
Zoorubin, 690
Zymase, 10, 11, 20, 21, 396
Zymogens, 15. See various enzymes.
Zymoplastic substances, 176, 228, 231

INDEX TO AUTHORS.

Abderhalden, E., protein hydrolysis, 29, 54, 55; carbohydrate group in the proteins, 33, 182; digestion products, 54, 55; histone, 62; elastin, 75; monanimo acids, 83, 84, 85, 88, 89, 91, 101; in the urine, 614, 675; cystine and cystinuria, 92, 631, 675; histidine, 99, 208; diamino trioxydodecanic acid, 101; proteoses in the blood, 183; blood serum, 188; globin, 208; blood corpuscles, 219; blood analyses, 238, 239; quantity of hæmoglobin, 239, 243; iron preparations, 245; blood and air dilution, 246; adrenalin, 278; sugar formation, 296; cholesterin, 334, 335; duodenal secretion, 377; trypsin, 394, 395, 417; protein absorption, 400, 413, 416, 417; ovalbumin, 507; milk, 529, 536; polypeptides in urine, 551, 614; glycylglycin, behavior in animal body, 630; Bence-Jones protein, 645; alcohol, 755; dipeptides from protein bodies, 35; spongin, 81
Abel, J., epinephrin, 278; spermin, 496; carbamic acid, 552; melanines, 689
Abeles, M., sugar, 118; uric acid, 186; liver sugar, 295; carbohydrates in the urine, 608
Abelmann, M., protein digestion, 418; fat absorption, 425
Abelsdorf, G., 490
Abelous, J., enzymes, 8, 17–20
Ach, L., 568
Achard, Ch., 185
Ackermann, D., 195
Adamkiewicz, A., protein reaction, 42; proteoses, nutritive value of, 746
Aducco, V., acidity of urine, 544; urine poison, 615
Aders, R. H., hydrolysis of gelatine, 78, 83, 84
Adler, O., pentose, 112; levulose, 119; blood, 217, 649
Adler, R., pentose, 112; levulose, 119; blood, 217, 649
Adrian, C., 594
Adriance, J., human milk, 531; colostrum, 531
Adriance, V., human milk, 531; colostrum, 531
Afanassiew, M., 333
Albanese, M., 579
Albert, R., 10
Albertoni, P., bile, 308; trypsinogen, 390; sugar, absorption of, 419
Albrecht, E., 193
Albro, A., 393
Albu, A., urine poisons, 615; mineral metabolism, 616, 625, 719, 735, 737
v. Aldor, L., 644
Aldrich, J. B., adrenalin, 278; skunk, secretion of, 692
Alexander, F., proteoses, 55, 522
Alexandroff, D., 102
v. Alfthan, K., carbohydrates of urine, 609; glucuronic acid in the urine, 667
Allard, E., 672
Allen, S., 359
Allihn, F., sugar determination, 290, 663
Almagia, M., amino acids and carbohydrate formation, 305; uric acid demolition, 574
Almén, A., sugar test, 116, 655; xanthine, 159; meat, 476; foodstuffs, 773
Aloy, J., enzymes, 8, 19
Alsberg, C., pseudonucleic acid, 46; nucleic acid, 154
Altmann, 147
Altmann, R., nucleic acids, 152, 156
Ambronn, H., 686
Amermann, G., pepsin digestion, 358; stomachic digestion, 370
Amiradzibi, S., 457
Amthor, K., fats, 135, 442
Andersson, J., 277
Andrlik, K., 89
v. Anrep, B., carbon monoxide hæmoglobin, 207; benzoic acid, 588
Anselm, R., 326
Ansiaux, G., protein coagulation, 40; fibrinogen and phosphorus poisoning, 172
Anthon, 115
Araki, T., methæmoglobin, 204; sulphurmethæmoglobin, 207; glycosuria, 299; nucleic acids, 395; lactic acid, 460, 461–463; in urine, 608; acetoacetic acid, 672; chitin, 686; chitosan, 687
Ardin-Delteil, P., 694

Argutinsky, P., meat, 476, 720; perspiration, 694
Armstrong, E. F., enzymes, 13, 16; osones, 107
Arnheim, J., 303
Arnold, J., 277
Arnold, V., neutral hæmatin, 208; acetoacetic acid, 672
Arnschink, L., 423
Arnstein, R., 581
Aron, H., colloids, 41; bone earths, 436, 438
Arrhenius, S., 190
Arronet, H., 238
Arteaga, T. F., 298
Arthus, M., blood coagulation, 170, 171, 226, 227, 229, 231; fibrinolysis, 174; glycolysis, 185; blood lipase, 185; peritoneal fluid, 231; sugar formation, 295; casein, 519; rennin coagulation, 520
Ascherson, 517
Ascoli, A., pyrimidine bases, 152, 164; nucleic acids, 152; plasminic acid, 156; protein absorption, 414; placenta, 512; urea formation, 550; uric acid demolition, 574
Asher, L., blood-sugar, 184; lymph, 250, 254–256; saliva, 340; protein absorption, 414; lactic-acid formation, 461
Aso, K., 19
Astaschewsky, 469
Athanasiu, J., blood coagulation, 234; fatty liver, 283; fat formation, 443
Atwater, W. O., metabolism, 471, 474, 716, 726, 748; respiration apparatus, 713, 722; alcohol in metabolism, 754; diets, 764, 767, 769
Aubert, H., 695
Austin, A. E., 290
Austrian, C. R., 571
Autenrieth, W., oxalic acid, 583; potassium, 623
Azémar, L., 670
Ayres, W. C., 489
Akermann, J., 365

Baas, H., cleavage of esters, 389; intestinal putrefaction, 586; demolition of tyrosine, 633
Babkin, B., oil soaps and pancreatic secretion, 385; urea formation, 551
Babcock, 523
Bach, A., autöxidation, 7; peroxides and oxidases, 7, 8, 17; enzymes, 8, 19
Baer, J., 544
Baeyer, A., assimilation, 1, 114; oxidations, 6; indol, 402
Baginsky, A., bile, 327; bones, 439
Baglioni, S., 240
Bainbridge, F. A., lymph formation, 255; lactase, 386
Baisch, C., carbohydrates in urine, 608; benzoylation, 659
Baker, J. L., 125
Balch, A., 307
Baldi, D., jecorin, 283, 481
Baldoni, A., 396
Balean, H., 216
Balke, P., purine bases, 159, 163; phosphocarnic acid, 457; episarkine, 580
Bang, B., 539
Bang, I., histones, 62, 269, 271; nucleic acids, 151, 155, 156, 270; leucocytes, 221; lymph glands, 269, 272; thymus, 270–272; parachymosin, 361, 362; proteoses in urine, 644
Banting, W., 770
Barbéra, A. G., lymph, 250, 254, 255; bile, 308; protein absorption, 414
Barbieri, I., phenylalanine, 91; isocholesterin, 337
Barker, L. F., 675
Barral, 185
Barratt, W., 695
Barth, H., 583
Bartoschewitsch, S. T., 589
Basch, K., casein formation, 537; milk, 538
Baserin, O., 332
Bashford, E., 586
Bassow, 349
Bastianelli, G., 378
Battelli, F., enzymes, 20, 21
Bauer, 605
Bauer, J., protein absorption, 412; fatty degeneration, 443
Bauer, M., 100
Bauer, R., 73
Baumann, E., diamines, 24; in urine and fæces, 615, 676; cystine and cystinuria, 93, 675; thiolactic acid, 94; carbohydrates, benzoylation of, 117, 659; iodothyrin, 276; diamidation, 305; putrefaction and products produced, 401, 586, 588; hippuric acid, 586; ethereal sulphuric acid, 588, 592, 637; phenol in urine, 590; pyrocatechin, 591; hydroquinone, 591; urine indican, 592, 593; bile acids, 597; oxyacids, 597; homogentisic acid, 597–600; carbohydrates in the urine, 608; sulphuric acid of the urine, estimation of, 622; sarcosin, destruction of, 631; demolition of compounds containing sulphur, 632; behavior of aromatic bodies, 633, 636, 639; mercapturic acids, 639; benzoyl cystine, 676
Baumann, K., 60
Baumgarten, O., 300
Baumstark, F., brain, 487; protagon, 481; cholesterin, 486; urinary pigments, 650
Bayer, H., 57
Bayliss, W. M., intestinal enzymes, 379; erepsin, 380, 391; enterokinase, 379, 383; trypsinogen and trypsin, 383, 386, 390, 392; secretin, 384
Beatty, W. A., 35
Beaumont, W., gastric fistula, 349; gastric digestion, 368
Beccari, L., 326

Bechamp, A., crystalline lens, 493; ovalbumin, 507; milk dextrin, 525
Bechhold, H., 656
Beck, A., urobilin, 603; urine poisons, 615
Beck, O, 471
Beck, R. F., 538
Beckmann, E., 692
Beckmann, W., 624
Becquerel, A., blood, 243; milk, 534
Beebe, S. P., 166
Beger, C., 536
Behrend, R., 568
Beier, K., 330
Beitler, C., 102
Bellamy, H., 385
Belloni, E., 523
Bence, J., 224
Bence-Jones, H, 645
Bendrix, E., pentoses, 72, 110, 111; glycogen formation, 292
Benedicenti, A., formalin protein combination, 49; alcohol in metabolism, 754; light and metabolism, 761
Benedict, F. G., metabolism in work, 471, 474; protein sparing, 748; alcohol in metabolism, 754
Benedict, H., elimination of sulphur, 471, 611
Benedikt, R., fats, 134, 137
Benrath, A., 364
Berard, E., protein coagulation, 40; ovglobulin, 506; ovalbumin, 507
Berdez, J., melanins, 688
Berenstein, M., 409
Bergell, P., carbohydrate groups in proteins, 33, 182; protein hydrolysis, 35, 395; amino acids, 92; lecithin, 147; adrenalin, 278; placenta, 512; glycylglycine, behavior in the body, 630; β-oxybutyric acid, 674
Berger, W. M., 344
Bergh, E., elastin, 75
v. der Bergh, 615
Bergin, T. J., 407
v. Bergmann, G., residual nitrogen, 183; taurine from cystine, 331
Bergmann, P., thyroidea, 276; pseudopepsin, 355; cæcum, 427
Bergmann, W., 620
Berlatzki, G. B., 401
Berlioz, A., 616
Berlinerblau, M., 241
Bernard, Claude, glycogen 148, 287; glycolysis, 184; sugar of the blood, 240; sugar formation, 295–298; diabetes, 299; pancreas, 382, 389; fat splitting, 389; fat absorption, 424; muscles, glycogen consumption in, 468
v. Bernek, M., 14
Bernert, R., oxyprotic acids, 31; monoxystearic acid, 131; pseudochylous, 262
Bernheim, A., transudates, 261, 263
Bernheim, R., 623
Bernstein, J., 477
Bernstein, N. O., 382
Bert, Paul, mammary gland, 514, 539; blood gases, 697, 706
Bertagnini, C., 636
Berthelot, M. P., fat splitting, 389; calorimetric determinations, 511, 724
Bertin-Sans, H., blood pigments, 204, 207
Bertrand, G., oxidation enzymes, 8, 18, 19; arsenic, 166, 275, 685; adrenalin, 278; epidermis formations, 685; poisons, 693
Bertz, F., 440
Berzelius, J. J., 347
Besbokaia, M., 385
Bethe, Alb., 486
Bial, M., pentoses, 110, 112; reagent, 667; conjugated glycuronates, 122, 667; diastase in the blood, 185, 295, 297; lymph, 251; glycogen formation, 294 sugar formation, 295
Bialobrzeski, M., 212
Bialocour, F., 371
Biarnès, G., oxidation enzymes, 8, 18
v. Bibra, E., 286
Bidder, F., buccal mucus, 341; saliva, 347; gastric juice, 353; pancreatic juice, 387; biliary fistula, 406; fat absorption, 424
Biedermann, W., 18
Biedert, Ph., 530
Biedl, A., 241
Biel, J., mare's milk, 529; human milk, 530
Bielfeld, P., 285
Binèt, P., 426
Bienstock, B., 408
Biernacki, E., alkalinity of blood, 223; blood analysis, 236; pepsin, 355; trypsin, 390; putrefaction processes, 405, 407, 588
Bierry, A., lactase formation, 386; maltase, 388
Biffi, N., 522
Bing, H. J., jecorin and blood sugar, 184, 283, 296
Binz, C., 631
Biot, J. B., 711
Biscaro, G., 523
Bischoff, Th., 716
v. Bittó, Bela, 671
Bizio, G., 695
Bizio, J., 287
Bizzozero, J., blood plates, 222, 227
Bjerre, P., 755
Blachstein, A., 459
Blankenhorn, E., protagon, 481, 482
Bleibtreu, L., blood analyses, 235; quantity of plasma, 238; urea estimation 561; protein requirement, 765
Bleibtreu, M., blood analyses, 235; quantity of plasma, 238; respiratory quotient, 446
Bleile, A. M., 240

Blendermann, H., tyrosine, behavior in the body, 305, 633; oxyhydroparacoumaric acid, 597

Bliss, C. L. 49

Blix, M. G., 236

Bloch, C., 587

Blondlot, N., 406

Blum, F., halogen proteins, 30; Millon's reaction, 42; protogen, 49; thyroid gland, 277; adrenalin glycosuria, 279

Blum, L., autolysis, 23; protein nitrogen, 27; cystine, demolition of, 631; proteoses, nutritive value of, 747

Blumenthal, F., acetone, 32, 668; nucleoproteids, 72, 282; pentoses, 110, 666; glycogen, 292; assimilation limit, 420; hippuric acid, 587; urine indican, 593

Boas, J., chymosin, 361; reagent for lactic acid, 374; for hydrochloric acid, 374

Bocarius, N., 496

Bock, C., blood sugar, 297; diabetes, 299

Bock, J., blood pigments, 206, 207; hæmoglobin and gases, 207

Bodländer, G., 754

Bodon, K., 260

Bodong, A., blood coagulation, 231; hirudin, 233

Boedeker, C., 591

Boedtker, E., urine nitrogen, 548; chlorine estimation, 617

Boehm, R., glycogen, 459, 466

Boehtlingk, R., 731

Boekelmann, W. A., 674

Bœmer, A., 60

Boeri, G., oxalic acid, 582; sulphur of urine, 611

Boettcher, 496

Boettger, 116

Bogdanow-Beresowski, 343

Bogomoloff, Th., 602

Bohland, G. W., 269

Bohland, K., urine nitrogen, 548; estimation of urea, 557, 561, 613; of ammonia, 625; uric acid elimination, 569; protein requirement, 765

Bohr, Chr., blood pigments, 196–200, 206; oxygen absorption, 199, 200, 704; oxygen tension, 706–708, 711; blood-gases, 696–698, 704; carbon dioxide tension, 710; swimming-bladder, 711; oxygen capacity, 711, 712; lungs, metabolism of, 704, 714; secretion of gases, 708, 710; egg, incubation of, 510, 512

DuBois-Reymond, E., muscle work, 469; smooth muscles, 477

DuBois-Reymond, R., 225

Bokorny, T., reserve protein, 5; carbohydrate formation, 114

Boldireff, W. N., intestinal enzymes, 378, 379; gastric digestion, 398

Boll, F., 489

Bonanni, A., bile, 326; conjugated glucuronates, 638

Bondi, J., 512

Bondi, S., sericin, 82; glycocholic acid, 312; taurocholic acid, 313; cholic acid, 316, 318; acetoacetic acid, 672

Bondzynski, St., oxyprotic acid, 31; koprosterin, 337; ovalbumin, 507; urinary purines, 578; oxyproteic acid in the urine, 612, 613; uroferric acid, 614

Bonnema, A., 517

Borchardt, L., diastatic enzyme, 295; acetone estimation, 673

Bordet, J., precipitins, 186; blood coagulation, 227, 231

Borissow, law of pepsin action, 358

Borissow, P., bitter principles ,350; allantoin, 584

Borkel, C., 57

Bornstein, K., work and protein metabolism, 471; protein feeding, 752

Boruttau, H., 459

Bosshard, E., 85

Bottazzi, Ph., sea animals, osmotic pressure, 189; erythrocytes, 220; intestinal protein, 379; heart muscles, 451; smooth muscles, 478; placenta, 512; tunicin, 686

Bouchard, Ch., autointoxication, 25, 280; glycogen formation, 291; poison of urine, 615

Boudet, 183

Boulud, conjugated glucoronates, 122, 184; pentoses, 184; virtual sugar, 184; maltose in urine, 667

Bouma, J., urinary indican, 595; reaction for bile pigments, 653; β-oxybutyric acid, 674

Bourcet, P., iodine, 187, 243; arsenic, 187, 243; menstrual blood, 243

Bourquelot, E., oxidation enzymes, 8; sugar elimination, 293

Bourquet, F., 191

Bovet, V., 30

Brahm, C., 638

Brand, J., bile, 307, 326, 327

Brandberg, J., 646

Brandenberg, K., alkalinity of the blood, 223

Brandl, J., 688

Brat, H., 112

Brauer, L., 329

Braunstein, A., glycolysis, 303; estimation of urea, 561

Bredig, G., enzymes, 11, 12; inorganic ferments, 14

Brenziger, K., 675

Brieger, L., ptomaines, 24; putrefaction products, 401; skatol, 409, 596; neuridine, 481, 486, 502; urinary indican, 592, 593; diamines in urine, 615; cystinuria, 676; perspiration, 694

Brinck, Julia, 415

Brion, A., 630

Brocard, M., 386

Brodie, T. G., fibrin ferment, 176; diabetes, 298; pancreatic casein, 396

Brook, F. W., 30

Brown, E. W., cholesterin esters, 183; allantoin, 574, 584

Brown, H., enzyme action, 14; isomaltose, 125; hydrolysis of starch, 128, 346; invertase, 379

Browne, C. A., (Jr.), 518

Brubacher, H., bones, 438, 439

v. Brücke, E., enzymes, 11; peptone, 52; coagulation of blood, 226; fibrin, 229; glycogen, 289, 290; pepsin, 355–357, in urine, 615; emulsification of fat, 398; absorption of proteins, 412; carbohydrates in the urine, 608

Brugsch H., 733

Bruhns, G., 164

Brunner, Th., 534

Bruno, G., action of steapsin, 389; action of bile and trypsin, 393, 397

Bruno, B., 616

Brunton-Blakie, 455

de Bruyn-Lobry, osamines, 107; varieties of sugar, 108

Bryant, A. P., diets, 767, 769

Buchanan, A., 175

Buchner, E., fermentation, 10, 11; zymases, 11, 21; gases of the lymph, 702

Buchner, H., fermentation, 10, 21

Budde, V., 664

Bülow, K., proteid, 38; starch, 126, 128

Bünz, R., 487

Bürger, L., 429

Bürker, K., 227

Buffalini, 244

Bugarsky, St., acid combinations of proteins, 38; blood serum, molecular concentration of, 189; degree of dissociation, 191; conductivity blood analyses, 236; urine, 546

Bulnheim, G., 316

v. Bunge, G., blood serum, 188; blood corpuscles, 220; blood plasma, 238; blood analyses, 237; blood and air dilution, 246; iron, of the liver, 285, 534; absorption, 245; secretion of hydrochloric acid, 364; gastric juice, 371; cartilage, 434; haematogen, 503, 510; milk, 527, 532, 535, 537; and growth, 535 536; hippuric acid, 586, 587; mineral requirements, 736; artificial feeding, 739

Bunsen, R., 561

Buntzen, J., blood in starvation, 244; blood and digestion, 244

Buraczewski, J., 215

Burchard, H., 336

Burckhardt, A. E., 188

Burian, R., xanthine oxidase, 18, 158, 273, 571; purine bases, 158, 163, 454, 470; in the urine, 579; formation of uric acid, 275, 569, 570, 573; destruction of uric acid, 574; histones in the urine, 647

Burow, R., 532

Busch, P. W., lymph formation, 254, 255

Busch, W., 368

Butlerow, A., 113

Cade, A., 352

Cahn, A., secretion of hydrochloric acid, 364; digestion products, 370; retina, 489

Camerer, W., milk, 526, 527, 531, 533–535; urinary nitrogen, 548; elimination of uric acid, 569; purine bases, 581; metabolism, 756, 577

Camerer, W., Jr., milk, 533, 535; elimination of ammonia, 624; perspiration, 693

Cammidge, P. J., 676

Campani, A., 621

Campbell, G., ovovitellin, 503; ovomucin, 506; ovalbumins, 507

Campbell, J. F., bilicyanin, 322, 324

Camps, R., 600

Camus, L., enterokinase, 383; secretin, 484; vesiculase, 496

Cannon, W. B., gastric movements, 366; food, digestibility, 369

Cappelli, J., erythrocytes, 220; smooth muscles, 278

Capranica, St., guanine, 161; perspiration, 694

Carlier, E. W., blood coagulation, 226; chyle, 251

Carl Theodore (Duke of Bavaria), 761

Carnot, Ad., bone earth, 436; dentin, 440

Carvallo, J., blood coagulation, 234; extirpation of the stomach, 369, 371

Casali, A., 692

Caspari, W., high altitudes, 246, 761; protein metabolism, 471, 750; milk fat, 538; vegetable diet, 750

Castaro, N., arginine, 97; hemicellulose, 130

Cathcart, E. P., glycogen formation, 294; absorption, 415, 417

Cavazzani, E., cerebrospinal fluid, 264; destruction of glycogen, 296; carbohydrate absorption, 421; phosphocarnic acid, 457; muscle work, 468; semen, 495

Chabbas, J., 491

Chabrie, C., 440

Chandelon, Th., 468

Chaniewski, 445

Chanoz, M., 284

Charcot, J. M., 496

Chassevant, A., 574

Chauveau, A., sugar formation, 306; fat formation, 444–446; muscle work, 468, 473

Chigin, P., 350

Chittenden, R. H., proteoses and peptones, 51, 52, 56, 60; keratin, 73; elastin, 75, 76; gelatine, 77, 79; saliva, 343–346; peptic digestion, 358, 359; gastric digestion, 369; tryptic digestion, 393; tendon mucoid, 428, 435; myosin, 450; neurokeratin, 73, 480, 488, 489; nutritive requirements, 766

Chodat, R., peroxides and peroxidases, 7, 8, 17, 19; enzymes, 8, 19

Chossat, Th., metabolism in starvation, 828, 732

Christenn, G., 531
Christensen, A., 646
Ciamician, G., 403
Cingolani, M., 568
Citron, H., 623
Clar, C., 569
Claus, R., 303
Claypton, J. L., 23
Clemens, Paul, conjugation of glucuronic acid, 638; Ehrlich's urine test, 675
Clemm, C. G., 533
Clerc, A., 185
Cleve, P. T., 316
Cloetta, M., hæmatin, 210, 212
Cloëz, 330
Clopatt, A., alcohol and metabolism, 754
Closson, O. E., 8
Cohn, Felix, 371
Cohn, M., saliva, 343; pancreas secretion, 387
Cohn, R., protein hydrolysis, 29; leucine, 85; leucinimide, 87; tyrosine, 89, 633; amino acids and carbohydrate formation, 306; amino benzoic acids, 636; nitrobenzaldehyde, 636; furfurol, 637; pyridine, 639
Cohn, Th., blood, 189; sperm crystals, 496; allantoin, 584
Cohnheim, J., ptyalin, 344; urine diastase, 616
Cohnheim, O., alcoholic fermentation, 21; proteins, 26, 38; glycolysis, 302, 303; erepsin, 378, 380, 416; pancreatic juice, 384; apparent feeding, 745
Cohnstein, J., 243
Cohnstein, W., alkalinity of the blood, 223; lymph formation, 256
Colasanti, G., metabolism of muscles, 467; lactic acid, 469, 608; xanthocreatinine, 567
Cole, S. W., protein reaction, 42, 43; tryptophane, 102, 103
Colenbrander, M., glycolysis, 185
Collmann, 695
Colls, P. C., 61
Comaille, A., lactoprotein, 523; milk, 537
Connstein, W., 185
Conradi, H., autolysis, 23, 281; antithrombin, 234; intestinal bacteria, 408
Constantinidi, A., foodstuffs, 421; vegetable diet, 750
Contejean, Ch., blood coagulation, 234; phlorhizin diabetes, 298; gastric juice, 353; acid secretion, 364; pyloric secretion, 365
Copemann, M., 649
Coranda, G., urea formation, 551; ammonia elimination, 624
Cordua, H., 216
Corin, G., protein coagulation, 40; fibrinogen, 172; ovoglobulin, 506; ovalbumin, 507
Coronedi, G., 442
Corvisart, L., 390
Le Count, E. R., 101
Courant, G., milk, 515, 520, 529; casein salts, 519
Cousin, H., 504
Couvreur, E., 291
Cramer, C. D., fibrinogen, 173; blood coagulation, 229
Cramer, E., fibroin, 82; sericin, 82; perspiration, 694
Cramer, Tr., 418
Cramer, W., protagon, 481; hippuric acid, 586;
Cremer, M., pentoses, 111, 290; glycolysis, 185; glycogen, 288–291, 293; phlorhizin diabetes, 298; sugar from glycerine, 308; fat formation, 445
Croftan, A., bile acids in blood, 238; suprerenal capsule, 279
Croft-Hill, A., 16
Croner, W., 375
Cronheim, W., 375
Croockewitt, J. H., 82
Cummins, G. W., trypsin digestion, 393; myosin, 450
Cunningham, R. H., 425
Curtius, Th., peptide syntheses, 34; cholic acid, 315
Cutter, W. D., tendon-mucin, 66, 428; saliva, 340
Cybulski, N., 278
Černy, T., lactic acid fermentation, 461
Czerny, A., 221
Czerny, F., alcoholic fermentation, 21
Czerny, V., extirpation of stomach, 369; absorption, 413
Czerny, Zd., 644

Daddi, L., fat in blood, 244; milk fat, 538
Daenhardt, C., 702
Dakin, H. D., arginase, 36, 97, 550; protamines, 63, 64, 84, 95, 97, 101; hexone bases, 98, 100; adrenalin substances, 278; ester cleavage, 284
Daland, J., 237
Danilewsky, A., protein sulphur, 28; plasteines, 56, 363; anti enzymes, 372, 379; muscle protein, 448, 451, 475; milk globules, 517
Danilewsky, B., 724
Danilewsky, W., 146
Dareste, C., testicles, 495; yolk of egg, 502
Darmstädter, E., 674
Darmstädter, J., 692
Darmstädter, L., 337
Dastre, A., fibrinogen, 172; fibrinolysis, 174, 175; blood coagulation, 227, 234; glycogen, 222, 251, 295, 296; liver, 282, 286; sugar elimination, 293; bile, 307, 324, 325; in stomach, 398; trypsinogen, 386; fat absorption, 424
Dautzenberg, P. J. W., 636
Dauwe, F., 12
Davis, W. S., 689
Day, H., 367
Dean, A. L., inulin, 127; protein regeneration, 417

Decaisne, E., 536
Dehn, W. M., 618
Delezenne, C., blood coagulation, 170, 226, 231, 234, 255; intestinal juice, 378; enterokinase and pancreatic juice, 379, 383, 386; secretin, 384
Delfino, A., 512
Demant, B., 378
Denigès, G., tyrosine, 91; uric acid, 576; determination of purine bases, 582; homogentisic acid, 600
Denis, P. S., 177
Derrien, E., methæmoglobin, 202, 205
Desgrez, A., 291
Deucher, P., pancreas, 418, 425
Devoto, L., determination of proteoses, 60, 61, 644
Devillard, P., 264
Diaconow, C., lecithin, 143, 147
Diamare, V., pancreas, 302, 381
Diels, O., cholesterin, 334, 335
Diesselhorst, G., 693
Dietschy, R., 644
Dietze, A., 393
Dillner, H., 506
Disqué, L., urobilin and urobilinogen, 601, 603, 604
Ditthorn, Fr., galactosamine, 65, 71, 121
Dittrich, P., 205
Dock, F. W., 299
Dörpinghaus, Th., carbohydrate groups in proteins, 33, 182; products of protein hydrolysis, 74, 84, 85, 88
Dombrowski, St., oxyproteic acids in urine, 613; uroferric acid, 614; urinary ptomaines, 615
de Dominicis, N., 300
Donath, J., 264
Donné, A., 651
Dormeyer, C., 137
Doyon, M., fibrinogen, 172; lipases, 185, 284; bile, 308, 309, 329; biliverdin, 324
Dragendorff, D., 652
Drechsel, E., oxidation, 8; protein substances, 26–29, 36, 38; gorgonin, 82; diaminoacetic acid, 97; hexone bases, 78, 98, 100; lysuric acid, 98; purine bases, 159; thyroidea, 276; jecorin, 283; urea formation, 550, 552; carbamates, 552; silicic acid ester, 685
Droop-Richmond, H., 517
Dubelier, D., 753
Ducceschi, V., blood coagulation, 235; heart-muscle, 451
Duclaux, E., protein coagulation, 40; milk fat, 518
Düll, G., 128, 129
Düring, Fr., 437
Dufourt, L., bile, 308, 309, 329; work, carbohydrate consumption in, 468
Duggan, C. W., 40
Dumas, J. A., 553
Dunlop, J. C., work and protein metabolism, 471; oxalic acid, 582
Ebstein, E., pentoses, 72, 110, 111
Ebstein, W., saliva, 345; pyrocatechin, 591; urinary calculi, 680; diet cures, 770, 771
Eckhard, C., 340
Edkins, J. S., pepsinogen, 364; pancreatic rennin, 396
ver Eecke, A., 756
Ehrenfeld, R., protein hydrolysis, 29; oxyglutaric acid, 32; halogen proteins, 32; leucine, 86; tyrosine, 90
Ehrenreich, M., 391
Ehrenthal, W., 409
Ehrlich, E., 719
Ehrlich, F., 87
Ehrlich, Paul, aldehyde reaction, 121, 675; precipitines, 186; bilirubin reaction, 321, 654; urine tests, 674
Ehrström, R., histone, 62; elimination of phosphorus, 620; peptonuria, 643
Eichholz, A., globulins, 180; glycoproteids, 506; urobilins, 602
Eichhorst, H., 413
Einhorn, M., 403
Eiselt, Th., 651
Ekbom, A., 317
Ekehorn, G., 619
van Ekenstein, Alberda, 108
Ekholm, K., 768
Ekunina, M., 461
Embden, G., cysteine and cystine, 28, 92, 94; proteoses in the blood, 183, 415; ethereal sulphuric acids, 280; conjugated glucuronic acids, 280; glycolysis, 303; amino acids, carbohydrate formation, 305; passage of, into urine, 614; protein regeneration, 416; lactic-acid formation, 416; acetone formation, 262
Embden, H., 598
Emerson, R. L., 394
Emich, Fr., 406
Emmerling, A., 74
Emmerling, O., enzymes, 12, 16, 17; reversion of sugar, 125
Ellenberger, W., gastric digestion, 370; protein absorption, 413; milk, 529; appendix, 397
Ellinger, A., isoserine, 96; diamines, 97, 98; diamino acids, 97, 98; indol-acetic acid and indol-propionic acid, 102; coagulation of bood, 234; lymph formation, 255, 256; pancreatic secretion, 387; urinary indican, 592–594; kynurenic acid, 600; proteoses, nutritive value of, 746
Elvove, E., 8
Ely, J., saliva, 343, 345
Engel, H., lipases, 363, 389
Engelmann, G. J., 471
Engler, C., 6, 7
Eppinger, 258
Erb, W., 38
Erben, Fr., oxystearic acid, 131; blood in disease, 246, 247; fat of chyle, 251; nitrogen partition in the urine, 549; urein, 563

Erlandsen, A. W. E., 143, 144
Erlanger, J., 427
Erlenmeyer, E., leucine, 85; tyrosine, 89, 90
Erlenmeyer, E. jr., tyrosine, 89; cystine, 93; serine, 96
Esbach, G., 645
Estor, A., 711
Etard, A., 457
Etti, C., 512
Eulenburg, A., 295
v. Euler, H., catalysis, 14, 15, 20
Eves, F., 345
Ewald, Aug., keratin, 73; gelatine, 78; hæmatoidin, 216; digestion of gelatine, 395; of cartilage, 395; visual purple, 489; corpora lutea, 498
Ewald, C. A., protein absorption, 413; gases of transudates, 703; metabolism and light, 761
Ewan, Th., 6
Eykman, C., isotony, 193; blood-corpuscles, 195; blood analysis, 236; inhabitants of tropics, 762
Eymonnet, 614

Fabian, E., 294
Falck, F. A., 744
Falck, C. Ph., 744
Falk, Ernst, 758
Falta, W., alcaptonuria, 598; protein metabolism, 719, 741; artificial nutrition, 738
Falloise, A., secretion of bile, 309; intestinal enzymes, 379; carbon dioxide tension, 710; metabolism, 761; lipase, 363
Fano, G., 171
Farkas, G., 191
Farkas, K., 512
Farnsteiner, K., 136
Farwick, B., 475
Faust, E., sepsine, 24; gelatine, 77; samandarin, 692
Favre, P. A., 694
Fawitzki, A., 554
Fedeli, C., 590
Feder, L., 551
Fehling, H., reagent, 116; sugar estimation, 660
Fehrsen, A., 244
Feinschmidt, J., 303
v. Fenyvessy, Bela, conjugated glucuronic acids, 610, 638
Fermi, Cl., fibrin, 175; foodstuffs, digestibility of, 368; autodigestion of the stomach, 372; trypsin test, 392
Fernet, E., 701
Filehne, W., 329
Filhol, 539
Fingerling, G., 536
Finkler, D., oxidations, 3, 723
Fischer, Emil, enzymes, 13, 16; protein hydrolyses, 29, 35, 54, 55, 74, 78, 82; peptides, 35, 55, 395; amino acids, 83–92, 101; ornithine, 97; diaminotrioxydodecanoic acid, 101; oxypyrrolidincarboxylic acid, 102; carbohydrates, 105–109, 113, 114, 120; glucosides, 108; sugar syntheses, 114; glucosamine, 107 108, 120; glucuronic acid, 121; isomaltose, 125; purine bases, 156, 157, 160–162; pyrimidine bases, 164, 165; trypsin, 394, 395, 416; lactose fermentation, 524; uric acid, 568; urinary purines, 579; conjugated glucuronic acids, 610
Fischer, Chr., 83
Fischler, M., 324
Flatow, R., 578
Flaum, M., 358
Fleckseder, R., 343
Fleig, C., bile, 309; pancreatic juice, 384; sapokrinin, 385
Fleischl, E., hæmometer, 219; formation of bile, 330
Fleitmann, 28
Fleroff, A., 63
Fletcher, H. M., 347
Flint, A., stercorin, 337; work and protein catabolism, 471
Florence, 496
Floresco, N., blood coagulation, 234; liver, 282, 286; biliprasin, 324
Flueckiger, M., urine, 609
Foa, C., saliva, 343; milk, 515, 530
Folin, O., animal gum, 67; alkalinity of blood, 223; muscle rigor, 466; acidity of urine, 545; urea, 561, 562; urein, 563; creatinine, 563, 565, 567, 744; uric acid, 569, 571, 578, 744; sulphur of urine, 611, 744; sulphuric acids of urine, 623; ammonia, 625; protein metabolism, 744
Fordos, M., 269
Forrest, J. R., 437
Forssner, G., 614
Forster, J., transfusion of blood, 248; mineral metabolism, 735; water and metabolism, 753; dietaries, 764, 770, 771
Fraenckel, P., blood, determination of alkalinity, 192; methods on blood analysis, 236; gastric juice, 354
Fraenkel, A., urine in phosphorus poisoning, 554; action of diluted air, 705; dyspnœa metabolism, 758
Fraenkel, Sig., proteins, 28, 33, 45; peptones, 53, 57, 60; thiolactic acid, 28, 94; histidine, 99; thyreoidea, 276; formation of glycogen, 294; gastric juice, 363; homogentisic acid, 599; chitin, 686
Framm, F., glutin, 79; sugar and alkali, 115; glycolysis, 185
Franchimont, A. P., 686
Frank, O., estimation of fat, 137; fat absorption, 421–423; curare poisoning, 467
Frankenstein, W., 6
Frankland, E., 724
Franz, Fr., 171
Frédericq, L., protein coagulation, 40;

serglobulin, 180; osmotic pressure, 190; hæmocyanin, 219; blood coagulation, 226; blood gases, 698, 706, 710
Frémy, E., muscles of cephalopods, 478; yolk-spherules, 509
Frentzel, J., glycogen, 288, 290; work and fat catabolism, 472, 474; nitrogen in meat and meat extracts, 477; calories and nitrogen, 727
Frerichs, F. Th., synovia, 266; human bile, 326; saliva, 347; uric acid, destruction of, 573
Freudberg, A., 223
Freund, E., serglobulin, 179; hæmatinogen, 216; coagulation of blood, 226; determination of chlorine, 617; starvation metabolism, 733; cellulose in consumptives, 714
Freund, O., 733
Freytag, Fr., cerebrosides, 268, 482, 483, 484; protagon, 481–483
Friedenthal, H., oxidases, 15; determination of alkalinity, 192; pepsin, 355; digestion of starches, 367; protein assimilation, 412
Friedjung, J. K., 533
Friedländer, G., 413
Friedmann, E., sulphur of proteins, 28; proteoses, 56; thiolactic acid, 74, 94; thioglycolic acid, 74; cystin, 93; taurine, 95, 321; adrenalin, 278; mercapturic acids, 639
Friend, W. M., erythrocytes, 194; pericardial fluid, 261
Fromm, E., 638
Fromme, A., 363
Frommer, V., 671
Frouin, A., gastric juice, 351; intestinal juice, 378; enterokinase, 383; pancreatic juice, 383
Fubini, S., respiration by skin, 695; metabolism and light, 761
Fürbringer, P., oxalic acid, 582; protein reagent, 643
v. Fürth, O., tyrosinase, 18; peroxyprotic acid, 31; xanthomelanin, 32; suprarenin, 278; bile and fat cleavage, 398; muscle proteins, 448, 451–453, 477, 478; muscle rigor, 466; smooth muscles, 477, 478; chitosan, 687; melanins, 689, 690
Fuld, E., fibrin formation, 176; blood coagulation, 231, 232, 234; chymosin, 361, 362; rennet coagulation, 520, 521
v. Funke, 471

Gabriel, S., cystine, 93; bones, 436; teeth, 440; ovalbumin, 507; asparagin, nutritive value of, 747
Gachet, J., 385
Gad, J., emulsification of fat, 398; myeline, 481
Gärtner, G., 219
Gaglio, G., lactic acid, 241, 460; oxalic acid, 629
Galdi, F., 258
Galeotti, G., 39
Galimard, J., 74
Gallois, 459
Gamgee, A., nucleoproteids, 72; nucleic acids, 153; oxyhæmoglobin, 200, 202; intestinal juice, 377; protagon, 481, 482; pseudocerebrin, 485
Ganassini, D., 343
Garnier, L., 598
Garrod, A. E., hæmatoporphyrin, 213–215, 650; homogentisic acid, 598–600; urochrome, 601; urobilin, 603, 605, 606; uroerythrin, 607; cystinuria, 676; diamines, 676
Gatin-Gruzewska, Z., seralbumin, 181; glycogen, 287, 288
Gaube, T., 694
Gaud, F., 115
Gaule, J., 245
Gautier, A., ptomaines, 24; leucomaines, 24, 457; xanthine, 157; arsenic, 166, 243, 271, 275, 685; menstrual blood, 243; thymus, 271; glycogen, 290; fat formation, 444, 445; muscles, 457; proteid of egg white, 507, 508; xanthocreatine, 457, 567; epidermis formations, 685
v. Gebhard, F., 467
Geelmuyden, H. C., acetone bodies, 669, 670; estimation of, 674
Geissler, 643
Gengou, O., blood coagulation, 227, 261
v. Genser, 534
Gentzen, M., tryptophane, 103; indol, 593
Geoghegan, E. G., cerebrin, 483, 484; brain, mineral constituents of, 489
Geppert, J., alkalinity of the blood, 223; oxygen of blood and air pressure, 705; respiration investigations, 713, 722, 733; alcohol, nutritive value of, 754
Gerard, E., reductases, 19; cholesterin, 335; uric acid fermentation, 568
Gerber, N., 531
Gerhardt, C., 672
Gerhardt, D., 604
Gerngross, O., 165
Gertner, W., 309
Gessard, C., 690
Geyer, J., 658
Geyger, A., 598
Giacosa, P., mucin substances, 66, 509; estimation of blood pigments, 217; frog's eggs, 509; urinary iron, 626; demolition of aromatic substances, 634
Giertz, H., 47
Gies, W. J., mucin substances, 66, 67, 68, 360, 428, 429, 435; elastin, 75, 76, 100; gelatine, 77, 100; hexone bases, 100; action of ions, 168, 169; lymph formation, 254, 255; pancreatic juice, 387; Achilles tendon, 429; ligamentum nuchæ, 428, 429; bones, 435; protagon, 482; ureine, 563
Gilbert, 455
Gilson, E., lecithin, 143, 147; chitin, 686
Ginsberg, S., 421
Githens, Th. St., 188

Gittelmacher-Wilenko, G., urine purines, 582; urine reducing power of, 609
Giunti, L., 630
Gizelt, A., 385
Glaessner, K., ethereal sulphuric acids, 280; gastric enzymes, 355, 362; Brunner's glands, 377; pancreatic juice, 387; protein absorption, 413, 416; kynurenic acid, 600
Glassmann, B., 561
Gleiss, W., 469
Gley, E., iodine, 187; coagulation of blood, 234, 235; lymphogogues, 255; enterokinase, 383, secretin, 484; vesiculase, 496
Glikin, W., 137
Gluzinski, A., 369
Gmelin, L., test for bile pigments, 321, 322, 652; saliva, 347
Gmelin, W., 353
Gogitidse, S., 538
Goldmann, E., cystine and cystinuria, 93, 675, 676; iodothyrine, 276; demolition of sulphurized compounds, 632; diamines, 675
Goldmann, H., 215
Goldschmidt, C., 556
Goldschmidt, F., 55
Goldschmidt, H., 344
Gonnermann, M., tyrosinases, 18, 90; glycocoll, 83; action of trypsin, 395
Goodbody, W., 401
Goodwin, R., 52
Gorodecki, H., 332
v. Gorup-Besanez, E. F., pericardial fluid, 260; human bile, 326
Gossio, B., 460
Gossmann, H., pancreas, 382
Goto, M., protamines, 63, 64; uric acid, 576
Gottleib, R., urea in blood, 240; iron in the bile, 326; in the urine, 626; urinary purines, 578; oxyproteic acid, 612
Gourlay, F., 275
de Graaff, C. J. H., 645
Graebe, C., 412
Graffenberger, L., 438
Graham, Th., 39
Grawitz, E., 246
Green, E. H., 81
Green, J. R., fibrinolysis, 174; blood coagulation, 229
Gregor, A., 567
Gregor, G., 623
Gréhant, N., urea in blood, 240, 242; urea formation, 553; fatty acids in the urine, 629
Griffiths, A. B., neurokeratin, 489; urine poison, 615
Grimaux, E., protein synthesis, 34, 234
Grimbert, L., 606
Grimm, F., 604
Grimmer, W., 367
Grindley, H. S., 767
Griswold, W., 345
Grober, J., 355
Groeber, A., 595
Grohé, B., 370
Groll, S., 244
Grosjean, A., 234
Gross, A., pseudochylous effusions, 262; ovovitellin, 503, 504
Gross, O., 625
Grosser, P., 596
Grouven, H., 475
Gruber, D., dextrins, 128, 129
Gruber, M., nitrogen deficit, 718; water and metabolism, 754
Grübler, G., 38
Grünbaum, D., 512
Gründler, J., 631
Grünhagen, A., 264
Grüns, G., 195
Grützner, B., 642
Grützner, P., estimation of pepsin, 357; gastric contents, 366; Brunner's glands, 377; pancreatic diastase, 388
Grund, G., pentoses, 72, 110, 111
Grutterink, 645
Gscheidlen, R., sulphocyanides, 343, 611; lactic acid, 460; urea formation, 553
Gubler, A., lymph, 252; witch's milk, 534
Gümbel, Th., 27
Gürber, A., seralbumin, 181, 182; serum, mineral constituents of, 187; alkalinity of blood, 224; bile, 329; amniotic fluid, 512
Guerin, G., 615
Günzburg, A., 374
Guillemonat, A., spleen, 274; liver, 285
Guinochet, E., 262
Gulewitsch, W., arginine, 96; choline, 145; nucleic acid, 154; thymin, 165; trypsin, 395; extractives of the muscles, 457
Gullbring, A., 314
Gumilewsky, 378
Gumlich, E., protein absorption, 413: urinary nitrogen, 548, 554; phosphorus metabolism, 619
Gunning, J. W., 670
Gusserow, A., 583
Guth, F., 132
Gyergyai, A., proteoses, 414, 746

Haas, K., 214
Habermann, J., proteins, 29, 32; amino-acids, 87, 88, 91
Händel, M., 434
Hänsel, E. W., 372
Häser, 627
Häusermann, E., 245
Hafner, A., 132
Hagermann, O., cutaneous respiration of the horse, 695; work and metabolism, 760
Hagen, W., 164
Hahn, M., fermentation, 10; digestion of casein, 521; Eck's fistula, 552; urea formation, 552, 553
Haig, A., 569
Haiser, F., 155

Haldane, J., blood pigments, 204–206; estimation of, 218; quantity of blood, 248; oxygen tension, 707, 708
Haliff, E., 20
Hall, W., purine bases, 163, 408, 454, 582; absorption of iron, 245; artificial nutrition, 739
Hallauer, B., 329
v. Hallervorden, E., urea formation, 551, 554; elimination of ammonia, 625
Halliburton, W. D., protein coagulation, 40, 61; nucleoproteids, 72; dextrins, 129; cell globulins, 141, 194; fibrin ferment, 176; seralbumin, 181; blood serum, 188; blood corpuscles, 194; blood coagulation, 234; tetronerythrin, 219, 690; pericardial fluid, 261; cerebrospinal fluid, 264, 488; liver, 281; glycogen, 289; pancreatic rennin, 396; myxœdema, 429; bone marrow, 437; muscles, 448–453; proteins of the brain, 479; diseases of the nervous system, 488; kidneys, 542
Halsey, J. T., 90
Ham, C. E., 216
Hamburger, C., amyolysis, 185, 345
Hamburger, H. J., theory of ions, 41; colloids, 169; blood serum, 187, 189, 190; stroma of blood-corpuscles, 192, 220; isotony, 193; permeability of the blood corpuscles, 195, 196; of the muscles, 465; leucocytes, 220; alkalinity of blood, 187, 224; lymph formation, 255; ascitic fluid, 263; intestinal juice, 377, 378; enterokinase, 383; absorption, 427
Hamburger, E. W., 326
Hammarsten, O., chymosin, 13; globulins 46, 179–181; nucleoalbumin, 47; mucin substances, 66, 67, 68, 509; helicoproteid, 71; nucleoproteids, 110, 282 381; protoplasm, 141; fibrinogen, fibrin and blood coagulation, 173, 176, 177, 183, 230; fibrin globulin, 177, 183; seralbumin, 181, 183; blood plasma and serum, composition of, 187; hæmatoporphyrin, 215, 650; casein, 179, 520; coagulation with rennet, 520; lymph gases, 251, 702; transudates, 258, 260–263; synovial fluid, 265; storage of carbohydrate, 294; bile mucin, 310; bile acids, 312, 314, 319; scymnol sulphuric acids, 310; dehydrocholic acid, 315; bile of various animals, 307, 312, 315, 319, 327; reaction for bile pigments, 232, 653; cholohæmatin, 324; human bile, 326, 327; phosphatides, 325, 328; urea in the bile, 325, 547; saliva, 345; antichymosin, 361; pancreas infusion, 391; pseudomucin, 500; perch-eggs, 504, 509; lactoprotein, 523; urinary protein, 645; phenylhydrazine test, 658
Hammerbacher, F., 347
Hammerl, H., 409
Hammerschlag, A., 222
Hanriot, M., enzymes, 13; lipases, 17, 185; diabetes, 300; respiratory quotient, 446; respiration, 713, 722
Hansen, C., lecithin, 143; protein regeneration, 417; fatty tissues, 441; yolk-fat, 504; milk-fat, 538
Hansen, W., 132
Hardy, W. B., 169
Harley, G., 600
Harley, V., liver and blood sugar, 297; intestinal putrefaction, 401; pancreas, 418, 425; large intestine, 426; urobilin, 604
Harms, H., 436
Harnack, E., ash-free protein, 38; iodospongin, 81; blood-pigments, 205, 207; hydramnion, 513; oxalic acid poisoning, 593; urinary sulphur, 611; demolition of chlorine substituted methanes, 631; gallic and tannic acid, 637
Harris, J. F., protein nitrogen, 27; nucleic acids, 152, 156; pyrimidine bases, 152, 156
Hart, E., protein nitrogen, 27; proteoses, 56; histidine, 99; hexone bases, 100
Hart, A. S., elastin, 75
Hartogh, 306
Hartung, C., 509
Hartwell, J. A., 52
Harvey, W., diet cures, 770, 771
Hasebroek, K., lecithin, 146, 404; pericardial fluid, 261; digestion products, 360
Haskins, H. D., ureine, 563; carbamic acid, 563
Haslam, H. C., salting out of proteins, 39, 179, 181; proteoses, 55, 56, 60
Hasselbalch, K. A., incubation of the egg, 510; absorption of oxygen, 704; oxygen tension, 711
Hauff, 534
Hausmann, W., nitrogen in protein substances, 27, 78; koprosterin, 338; cholesterin, 337
Hawk, P. B., bones, 435; water and metabolism, 754
Hay, M., 262
Haycraft, J. B., protein coagulation, 40; blood coagulation, 171, 226; diabetes, 300; biliverdin, 323; test for bile-acids, 652
Hayem, G., hæmatoblasts, 226; pernicious anæmia, 246; estimation of hydrochloric acid, 376
Hedenius, J., gizzard, 74; bile pigment, 325
Hedin, S. G., elastin, 75; protein hydrolysis, 78; hexone bases, 96, 98–100; blood-corpuscles, 193–195; blood analysis, 236; hæmatocrit, 237; lienases, 273; action of trypsin, 392; enzymes of the muscles, 454
Hédon, E., pancreatic diabetes, 301; absorption of sugar, 419; of fat, 425

Heffter, A., liver, 283; muscle reaction of, 447, 466; lactic acid, 463, 469; hyposulphites in the urine, 612; foreign bodies in the urine, 629; synthesis of hippuric acid, 635
Heger, P., 280
Heidenhain, M., 44
Heidenhain, R., lymph, 250, 253–255; transudates, 257; bile secretion, 307, 308; saliva, 341, 437; stomach, 349, 350, 363, 365; pyloric secretion, 365; pancreas and its secretion, 381, 382; trypsin, 386, 391; protein regeneration, 416; sugar absorption, 420; smooth muscles, 477; milk, 537
Heilner, E., 754
Heinemann, H. N., 474
Heintz, W., fat, 133; lactic acid, 460; estimation of uric acid, 569, 576
Heiss, E., 439
Heitzmann, C., 439
Hekma, E., intestinal juice, 377–379; enterokinase, 383; trypsinogen, 386
Hele, T. Sh., 599
Helier, H., 609
Heller, Fl., protein test, 41, 641; uroxanthine, 592; urinary pigments, 601; blood test, 649; urinary calculi, 683, 684
Helm, 349
Helmholtz, H., 470
Hellwig, smooth muscles, 477
Hemmeter, J. C., 407
Henderson, Y., protein nitrogen, 27; protein regeneration, 416, 417
Henkel, Th., 518
Henneberg, W., 397
Henninger, A., 60
Henocque, A., 219
Henri, V., enzymes, 13, 14; oxyhæmoglobin, 199
Henriques, R., 138
Henriques, V., lecithin, 143; lecithin-sugar, 184; blood, reducing substance, 240; protein regeneration, 417; adipose tissue, 441; fat of yolk, 504; milk-fat, 538; blood gases, 697; lungs, oxidation, 704
Hensen, V., lymph, 253; lymph-gases, 702
Henze, M., gorgonin, 82; iodogorgonic acid, 82; aspartic acid, 88; hæmocyanin, 219; copper in cephalopods, 286; spongosterin, 337; extractives of muscle, 455; muscles of octopods, 478
Heptner, F. K., 327
l'Héritier, 534
Herlant, L., nucleic acids, 154–156
Hermann, L., muscle work, 9, 466; hæmoglobin in starvation, 244; formation of fæces, 409; muscle rigor, 466; allantoin, 583
eron, J., amylolysis, 128, 344; invertin
Hin the intestine, 378
Herrmann, Aug., trypsin, 394; uric acid, 569
Herrmann, E., 166
Herter, E., saliva, 347; ethereal sulphuric acids, 589, 591, 637; oxybenzoic acids, 636; oxygen tension, 706
Herth, R., proteosis and peptones, 52, 60
Hervieux, Ch., indol and indican, 186, 592–594; skatol red and urorosein, 596; uroerythrin, 607
Herzen, A., spleen and digestion, 274; secretion of gastric juice, 350; charging theory, 365–385; pancreatic juice, 384, 385
Herzfeld, A., 107
Herzog, R. O., zymases, 11; histidine, 100
Hesse, A., 296
Hetper, J., hæmin, 212; hæmopyrol, 215
Heubner, O., casein, utilization, 530; nurslings metabolism, 752
Heubner, W., fibrinogen, 177; mytolin, 451
Heuss, E., 693
Hewlett, A. W., 427
Hewlett, R. T., 40
Hewson, W., 226
Heymann, F., pseudomucin, 500
Heymans, J. F., 481
Heynsius, A., salting out of proteins, 51; bilicyanin, 322, 324
Hiestand, O., 144
Hilbert, P., 634
Hildebrandt, H., oxalic acid, 583; amino-benzoic acid, 636; conjugations with glucuronic acid, 638
Hildebrandt, P., mammary glands, 514, 537; conjugations with glucuronic acid, 610
Hildesheim, 764
Hilger, 69
Hilger, A., 458
Hiller, E., 438
Hirsch, R., glycolysis, 303; amino-acids in the urine, 614
Hirschberg, A., 222
Hirschfeld, E., 371
Hirschfeld, F., work and nitrogen excretion, 471; uric acid, 569; acetone bodies, 669; metabolism, protein, 738, 750, 765; nutritive requirements, 770
Hirschfeld, H., 643
Hirschl, J. A., 658
Hirschler, A., putrefaction processes in the intestine, 405, 407, 589; ethereal sulphuric acids, 589
His, W., 434
His, W., Jr., purine bases, 164; uric acid, 575; pyridine, 639
Hlasiwetz, H., protein bodies, 29; amino-acids, 87, 88, 91
Hochhans, H., 245
Hoeber, R., colloids, 169; osmotic pressure, 189; determination of alkalinity, 191; permeability of the blood-cor-

puscles, 195; of the muscles, 465; absorption, 427; acidity of the urine, 545
Höne, J., 652
Hofbauer, L., 343
van't Hoff, J., 6
Hoffmann, A., 586
Hoffmann, F. A., transudates, 259, 261; œdematous fluid, 265; blood-sugar, 297; diabetes, 299
Hoffmann, J., 544
Hoffmann, P., 626
Hofmann, tyrosine test, 91
Hofmann, Fr., fat and fat formation, 441-443
Hofmann, K. B., muscles, 474; brain, 486
Hofmeister, F., cellular enzymes, 23; protein nitrogen, 27; halogen proteins, 30; glucosamine in proteins, 33; amino-acids, 3; bindings, 34; removal of proteins, 44; synproteose, 55; collagen, 77; gelatine, 79; serglobulin, 178, 180; pus, 267; gastric movements, 366; protein absorption, 414, 416; assimilation limit, 420; ovalbumin, 507; crystallization of proteins, 507; urea formation, 553; creatinine, 564; globulin in urine, 646; lactose, 666
Hofmeister, V., gastric digestion, 370; cellulose, 397; protein absorption, 413
Holde, D., 132
Holmgren, E. S., submaxillary glands, 340; phenylhydrazine test, 658
Holmgren, Fr., blood-gases, 697; elimination of carbon dioxide, 710
Holmgren, J., muscle stroma, 448, 451
v. Holst, G., mucins, 66, 265; transudates, 258
v. Hoogenhuyze, C. J., creatinine, 563, 567
Hooker, D., 255
Hopkins, F. G., halogen proteins, 30; Adamkiewicz's reaction, 42; tryptophane, 102, 103; protein crystallization, 182, 507, 508; uric acid, 569, 577; urobilin, 603, 605, 606; butterflies, 690
Hoppe-Seyler, F., oxidations, 3; cleavage processes, 5; proteins, 36, 47; compound proteids, 65; collagen, 76; protoplasm, 141, 145, 148; lecithins, 143, 145, 147; glycogen, 148, 287; cholesterin, 148; nuclein, 149; xanthine, 160; blood-plasma, 187; blood-corpuscles, 194, 219; blood-pigments, 196, 197, 198, 202, 204-210, 213, 214, 217; urobilinoids, 214, 603; blood analysis, 237; chyle, 251; pericardic fluid, 260; pus, 267-269; cerebrin, 268; struma, 275; bile, 326, 329; excretin, 410; cartilage, 434; bones and teeth, 437, 440; lactic acids, 461, 463, 469, 608; retina, 489; ovovitellins, 47, 503; casein, 518; milk, 526; bile acids in urine, 652; inosite, 668; chitosan, 687; sebum, 691; respiration apparatus, 712, 722
Hoppe-Seyler, G., determination of blood pigments, 217; elimination of phenol, 590; of indoxyl, 592-595; urobilin, 604, 606
Horbaczewski, J., keratin, 73; elastin, 75, 76; glutamic acid, 88; purine bases, 159, 160; uric acid, 568; formation of uric acid, 274, 570; urostealith, 682; metabolism, 739
Homborg, A. J., gastric juice, 352, 353
Horne, R. M., 171
Horodynski, W., 241
Hougardy, A., 181
Howell, W. H., 464
Huber, A., blood coagulation, 171; fibrinolysis, 174; liver diastase, 295
Huebner, R., 403
Hüfner, G., leucine, 85; blood-pigments, 197, 198, 199, 203-206, 209, 218; spectrophotometry, 218; dissociation of oxyhæmoglobin, 199, 704; glycocholic acid, 313; bile, 313; bird's eggs, gases of, 509; urea determination, 562; oxygen tension, 706; gases of the swimming-bladder, 711
Hürthle, K., 183
Hugounenq, L., biliverdin, 324; hæmatogen, 503; ash of child and milk, 535
Huiskamp, W., fibrinogen and fibrin formation, 173, 174, 175, 176, 177; nucleoproteids, 178, 270, 271; thymus histones, 270, 271
Hultgren, E. O., foodstuffs, 417, 421; appendix, 427; diets, 764, 767
Humnicki, V., koprosterin, 337
Hundeshagen, F., sponges, 81; lecithin, 143
Hupfer, Fr., 586
Huppert, H., digestion products, 55; glycogen, 221, 288; reaction for bile pigments, 322, 653; estimation of pepsin, 357; meat, 476; urea, 555; uroleucic acid, 600; urine, estimation of proteins, 646; laiose, 665; determination of acetone, 673
Hurtley, W. H., 600
Husson, 539
Hybbinette, S., 608

Ide, 457
Ignatowski, A., 675
Ikeda, K., 14
Inagaki, C., 181
Inoko, Y., 198
Inonye, K., intestine nucleic acid, 151, 154; cytosine, 165; lactic acid in urine, 608
Inonye, Z., 364
Irisawa, T., lactic acid, 241, 608
Ito, M., 643
Iversen, A., secretion of prostate, 496; concretions, 498
Iwanoff, 621
Iwanoff, L., nucleases, 153, 394

Jaarsveld, G. J., 588
Jablonsky, J. 387

Jackson, H. C., lactic-acid formation, 461; kynurenic acid, 600
Jacobsen, A., 184
Jacobsen, O., human bile, 326, 327; behavior of aromatic substances in the body, 635
Jacobson, J., 19
Jacoby, M., oxidation enzymes, 8, 18; autolysis, 22, 281, 713; phosphorus poisoning, blood in, 172, 175; demolition of uric acid, 574
Jacubowitsch, 347
Jaeckle, H., human fat, 132, 134, 441; fat determinations, 136; lipomia, 442
Jäderholm, A., blood pigments, 204, 209
Jaeger, A., 711
Jaffa, M. E., diets, 767, 769
Jaffé, M., ornithin and ornithuric acid, 97, 635; bile-pigments, 322; creatine, 456; phenylsemicarbazide, 556; urethane, 563; creatinine, 565, 567; uric acid, 572, 575; urinary indican, 592–594; kynurenic acid, 600; urobilin, 409, 601, 602, 604, 605; behavior of aromatic substances, 633, 635–638; furfurol, 637; thriophenic acid, 637
v. Jaksch, R., alkalinity of blood, 223; urea in the blood, 240; brain, 480; urine, partition of nitrogen in, 549; volatile fatty acids, 608; melanin, 651; phenylhydrazine test, 657; pentosuria, 666; acetone, 668
Jakowsky, M., 400
Jalowetz, E., 125
Jamieson, G. S., 82
Jantzen, F., 537
Jaquet, A., oxidation enzymes, 8; hæmoglobin, 198; estimation of blood pigments, 219
Jastrowitz, M., pentoses, 110, 666
Jelinek, J., fermentation, 21, 461
Jensen, P., 459
Jerome, W. Smith, 612
Jesner, S., 491
Joachim, J., serglobulin, 179; transudates, 258, 261, 263
Jodlbauer, 436
Johansson, J. E., serglobulin, 182; exchange of material and gas, 474, 759–762, 768
Johnson, E. G., chymosin, 361
Johnson, St., creatinine, 563–566
Johnson, T. B., triticonucleic acid, 156; cytosine, 165
Jolin, S., pig-bile, 313; elimination of ammonia, 624
Jolles, A., blood-catalases, 20; oxidation of protein, 32; determination of iron, 217; bile-pigments, 321, 653; milk, 533; urinometer, 547; uric acid, 578; urobilins, 602; urine, iron of, 626; protein of, 642; nucleohistone, 647
Joly, 539
Jolyet, 697
Jones, W., autolysis, 22; nucleoproteids, 72; nucleic acids, 153; enzymes of nucleic acids, 158, 273, 571; thymine, 165; nucleases, 271; thymus, 271; spleen, and uric acid, 273, 275
de Jonge, D., 693
Jorissen, W. P., 6
Jornara, D., 693
Josephsohn, A., 600
Jowett, H. A. D., 278
Jünger, E., 318
Jungfleisch, E., 463
Justus, J., 166
Juvalta, N., 633

Kalberlah, F., 670
Kanitz, A., steapsin, 389; trypsin, 393
Kareff, N., 173
Kast, A., intestinal putrefaction, 407; urine sulphur, 611; elimination of chlorine, 616; methane substituted by halogens, 631; demolition of sulphurized substances, 632; sweat, 694
Kastle, J. H., enzymes, 7, 8, 16
Katsuyama, K., lactic acid, 241, 461; inanition, 731
Katz, A., 604
Katz, J., 477
Katzenstein, A., 630
Katzenstein, G., 760
Kauder, G., globulin, 180; seralbumin, 181
Kaufmann, M., glycogen, 295; sugar of the blood, 297; sugar formation from fat, 306; fat formation, 444–446; respiratory quotient, 446; muscles, urea of, 455; sugar consumption of, 468; urea formation, 554; methods of metabolism investigations, 723
Kaufmann, M., urine purines, 579
Kaufmann, Martin, hunger, protein catabolism in, 730; gelatine, nutritive value of, 746
Kaup, J., 471
Kausch, W., 293
Keller, A., 614
Keller, W., 635
Kellner, O., foodstuffs, utilization of, 418; work and protein, 471; asparagin, 747; diets, 764
Kelly, A., extractives of muscles, 455; chitin, 686
Kermauner, F., 409
Kerner, G., 564
Kiliani, H., 105
Kirschmann, J., 746
Kirk, R., 600
Kisch, F., 459
Kishi, K., 277
Kistermann, 658
Kitagana, F., 485
Kjildahl, nitrogen determination, 556, 557
Klages, A., 318
v. Klaveren, H. K. L., 208

Klecki, K., 409
Kleine, F., urinary sulphur, 611; formanilide, 634
Klemensiewicz, R., stomach, formation of acid, 363; pylorus secretion, 365
Klemperer, G., chymosin, 361; urochrome, 601; oxalic acid, behavior of, 630; metabolism of protein, 738, 750, 765
af Klercker, O., 563
Klingemann, F., 539
Klingenberg, K., 634
Klug, F., tryptophane, 102; pepsin and pseudopepsin, 354; products of digestion, 360; trypsin, 390; elimination of phosphoric acid, 620
v. Knaffl-Lenz, E., 286
Knapp, K., sugar test, 116; determination of sugar, 660, 662
v. Knieriem, W., digestion of cellulose, 397; formation of urea, 550, 551; formation of uric acid, 572
Knöpfelmacher, W., human fat, 132, 441
Knoop, F., histidine, 99; methyl imidazole, 108; proteoses in the blood, 183, 415; protein regeneration, 416; demolition of aromatic substances, 633, 634
Knop, W., 562
Kobert, H. U., 196
Kobert, R., haemolysis, 193; cyanmethæmoglobin, 205; urinary iron, 626; melanines, 689
Kobrak, E., 531
Koch, W., lecithans and lecithins, 146, 148, 487, 488; neurokeratin, 480; protagon, 482; cephalin, 485, 486, 488; cholesterin, 480; brain analysis, 487; milk, 532
Kocher, Th., 276
Kochs, W., ethereal sulphuric acids, 280; hippuric acid, 586
Koefoed, E., 518
Köhler, A., meat 476; calorific value, 727; asparagin, 447
König, J., muscle, 475; milk, 527–529, 638; foods, 773
Köppe, H., blood corpuscles, 192–196, 220; volume determination, 237; isotomy, 192, 193; hæmoglobin, 193, 194; secretion of hydrochloric acid, 364
Köster, H., 521
Koettgen, E., 490
Kohlrausch, Fr., 191
Kolisch, R., blood, 240; creatinine, 567; urinary histone, 647
Koraen, G., muscular activity, 474; food and metabolism, 762
v. Koranyi, A., blood, 224; osmotic pressure, 256
Kossel, A., nitrogen of the proteins, 27; protein hydrolysis, 29, 78; arginase, 36, 97, 550; protamines, 59, 61–64, 84, 96, 98, 498; histone, 62, 63, 96, 97; nucleoproteins, 71, 72; aminovalerianic acid, 84; serine, 95; hexone bases, 96, 98–100; α-proline, 101; saponification, 136; the cell, 140, 141, 142; nucleohistone, 141, 270; nucleic acids, 152–156, 498; pyrimidine group, 152, 164–166; plasminic acid, 156; purine bases, 152, 156, 158, 161–164, 271, 284, 382, 454; hæmoglobin, 197; blood plates, 222; cerebrosides, 268, 482–485; protagon, 481, 482; ichthulin, 504
Kossler, A., phenol determination, 589, 590
Kostin, S., 206
Kostytschew, S., nucleic acid, 153, 155
Kotake, Y., nucleic acid of the intestine, 151, 154; cytosine, 165; vanillin, 635
Kotliar, E., 280
Kowalewsky, K., products of digestion, 53; uric acid, 572
Kowarski, A., 455
Kramm, W., 565
Kranenburg, W. R. H., 364
Krarup, J. C., 761
Kratter, Jul., 442
Kraus, Fr., fatty liver, 283; amino acids and formation of carbohydrates, 305
Krauss, E., 594, 595
Krawkow, N. P., amyloid, 69, 70, 431; chitin, 686, 687
Krehl, L., 647
Kreis, H., 132
Kresteff, S., 365
Kreusler, W., protein substances, 29
Kreuzhage, C., 471
Krieger, H., protein substances, 38; seralbumin, 181, 182; phosphaturia, 621
Krimberg, R., 457
Kröber, E., 111
Krönig, B., 168
Krogh, A., oxygen in the blood, 704, 711
Kronecker, F., 588
Krüger, A., denaturization of proteids, 40; hæmoglobin, 201; leucocytes, 221; blood, 221, 241, 242; spleen, 274; liver, 286; saliva, sulphocyanide, 343; intestinal juice, 377
Krüger, M., saponification, 136; alloxuric bases, 156, 159, 163; in fæces, 408; in urine, 578–581; ammonia, 625
Krüger, Th. R., peptones, 58, 80; phosphocarnic acid, 457, 474
Krug, B., 752
Krukenberg, F. C. W., hyalogens, 68; keratin proteoses, 73; skeletins, 81, 82 lipochromes, 186; hæmerythrin, 219; extractive bodies of muscle, 455, 456; bird's eggs, 509; urostealith, 682; bird's feathers, 690
Krummacher, O., determination of fat, 137; activity, work, metabolism, 471; heat of combustion, 728; gelatine, its nutritive value, 746
Krumbholz, C. J., 422
Kübel, F., saliva, 345, 346
Kühling, O., behavior of aromatic substances, 637, 638

Kühne, W., enzymes, 10; proteose and peptones, 51–60, 359; gelatine, 78; tyrosine, 89; paraglobulin, 178; hæmatoidin and lutein, 216; glycogen, 287; gastric digestion, 367, 371; the pancreas and its enzymes, 387–391, 395, 396; emulsion of fat, 422; proteins of the muscles, 448, 449; smooth muscles, 477, 478; neurokeratin, 73, 480, 489; pigments of the eye, 490, 491; lens, 493; corpora lutea, 498
Külz, C., 259
Külz, E., cystine, 92; pentoses, 110, 666; amylolysis, 125, 344, 389; glycogen, 287, 288, 291, 295; diabetes, 298, 300; gastric juice, 364; muscle activity, 468; gases in milk, 533; conjugated glucuronic acids, 632, 638; oxybutyric acid, 673
Külz, R., methæmoglobin, 204; determination of glycogen, 290; gases of the saliva, 342, 702
Kueny, L., 117
Küster, W., blood-pigments, 209–212; hæmatinic acids, 211, 320; hæmopyrrol, 214; bile-pigments, 210, 320, 321, 323, 324
Kuhn, 491
Kuliabko, A., pancreas, 302; ureine, 563
Kumagawa, M., formation of fat, 444; determination of sugar, 663; protein metabolism, 738, 750, 765
Kunkel, A. J., arsenic, 166; carbonmonoxide blood-test, 206; iron preparations, 245; iron and bile, 326, 332; urinary iron, 626
Kuprianow, J., 460
Kurajeff, D., halogenic proteins, 30; plasteine, 56; protamines, 63; tryptophane, 102
Kurbatoff, D., 135
Kurpjuweit, O., 407
Kusmine, K., 255
Kutscher, Fr., nitrogen of the proteins, 27; products of digestion, 54, 57; histones, 61, 63, 88, 89; protein hydrolysis, 78, 88, 89; hexone bases, 96–100; nucleic acids, 154; martamic acid, 154; cytosine, 166; thymine, 271; erepsin, 380; guanidine, 394; choline, 394; intestinal contents, 400; absorption of protein, 413; bases of meat extracts, 457; gelatine, oxidation, 582; neurine, 615
Kuwschinski, P., 387
Kyes, P., lecithin, 146; in hæmolysis, 193

Laache, S., 246
v. Laar, J., 73
Ladenburg, A., 496
Laidlaw, P. P., blood-pigments, 216; turacin, 690
Lanceraux, 301
Landauer, A., bile and putrefaction, 406; water and metabolism, 734, 754
Landergren, E., foodstuffs, 417, 421; metabolism, 730, 738, 748; sugar formation, 749; diets, 764, 767
Landois, L., stroma fibrin, 195; transfusion, 248
Landolt, H., 688
Landwehr, H., animal gum, 67, 525, 608
Lang, G., 646
Lang, J., taurine, 95; lymph, 253
Lang, S., deamidation, 305; lactic acid formation, 572; nitriles, 631
de Lange, C., milk, 533, 535
Langendorff, O., 464
Langer, L., 134
Langgaard, A., 530
Langhaus, Th., 216
Langley, J. N., saliva, 345, 347; propepsin, 364; cells of the gastric glands, 365
Langstein, L., carbohydrate from protein substances, 33, 65, 180, 181, 506; digestion products, 53, 57; scatosine, 103; proteoses in the blood, 183, 415; residual nitrogen, 183; serum, 188; animo acids, deamidation, 305; ovoglobulin, 506; ovalbumin, 507; ovomucoid, 508; alcaptonuria, 598, 600; kynurenic acid, 600; urinary quotient, 628, 720; lactic acid in the urine, 665
Lankester, E. R., 219
Lannois, E., absorption of sugar, 419; cerumen, 592
Lapicque, L., spleen, 274; liver, 285, 286; diets, 767
Lappe, J., 379
Laptschinsky, M., 493
Laqueur, E., casein and rennet coagulation, 518–521; paracasein, 521; lipase, 363
Larin, A. M., 358
Lassaigne, J. L., 583
Lassar, O , 223
Lassar-Cohn, bile acids, 315, 317; human bile, 318; myristic acid in the bile, 325
Latarjet, A., 352
Latham, 4
Latschenberger, J., blood, 170; bile-pigments, 330, 332; iron of liver, 332; protein absorption, 413
Latschinoff, P., bile-acids, 317
Laulanié, F., respiratory quotient, 446; work and sugar consumption, 473, 760
de Laval, G., 526
Laves, E., diabetes, 300; milk-fat, 530
Laves, M., 295
Lavoisier, A. L., 759
Lawrow, D., digestion products, 53; histones, 61; histidine, 99; blood-pigments, 208; conjugated glucuronic acids, 638
Lawrow, Maria, 56
Lawes, 445
Laxa, O., casein and rennet coagulation, 519, 521
Lazarus-Barlow, W. S., 255
Lea, Sh., 346

Leathes, J. B., autolysis of spleen, 273: fat formation, 297; proteosis, absorption of, 415, 417; ovarial fluid, 500
Lebedeff, A., liver fat, 282; fat of food, 442
Leclerc, A., 694
Leconte, P., 351
Ledderhose, G., glucosamine, 120; chitin, 686
Ledoux, A., 234
van Leersum, E. C., pentoses, 112; conjugated glucuronic acids, 122
Legal, E., indol, 402; acetone, 671
Lehmann, C., fat formation, 445; metabolism in starvation, 729; in work, 760
Lehmann, C. G., saliva, 347; protein of egg-white, 506
Lehmann, Fr., 695
Lehmann, K. B., hæmorrhodin, 207; adipocere, 442; sugar determination, 663
Leichtenstern, O., blood, 243, 244
Leick, 441
Leipziger, R., 719
Lelli, G., 480
Lemaire, F., isomaltose, 608; and lactose in the urine, 665
Lemus, W., 518
Leo, H., fatty liver, 282; diabetes, 300; acids of gastric juice, 375, 376; fatty degeneration, 443; laiose, 665; nitrogen deficit, 718
Lepage, L., 384
Lépine, R., conjugated glucuronic acids, 122; pentoses, 184; virtual sugar, 184; glycolysis, 185, 251, 302; glycogen, 291; absorption, 419; sulphur of urine, 611, 612; phosphorus compounds in the urine, 614; urine poison, 615; maltose in the urine, 667
Lepinois, E., 616
Lerch, 493
Lesem, W., 482
Lesnik, M., 633
Lesser, E. J., 417
Lesser, K. A., 254
Leube, W., urine, 640; sweat, 694
Leuchs, H., serin, 95; oxypyrrolidine carboxylic acid, 102; glucosamine, 107, 108, 120
Levene, P. A., autolysis, 22, 495; pseudonucleic acid, 46; proteoses, 56; tendon-mucin, 66, 428; hydrolysis of gelatine, 78, 79; amino acids, 83; nucleic acids, 151-156, 514; pyrimidine bases, 152, 154, 156, 394; nucleoproteids, 272, 479; glucothionic acids, 222, 273, 285, 431, 514; phlorhizin diabetes, 298; trypsin, 390; ichthulin, 504; dipeptides, 35
Levites, S., 27
Levy, A. G., 223
Levy, H., 637
Levy, L., 454
Levy, M., 439
Lewandowski, M., 412
Lewin, K., uric acid and hippuric acid, 586; indican, 593
Lewin, L., hæmoverdin, 208; hydroquinone sulphuric acid, 591
Lewinsky, J., 187
Lewis, Th., 478
Lewy, B., 496
Lewy, Benno, 227
v. Leyden, E., 496
v. d. Leyen, E., 593
Lieben, A., hæmatoidin and lutein, 216; iodoform test, 670
Liebermann, C., cholesterin, 336; shell of bird's eggs, 509; carminic acid, 690
Liebermann, L., catalases, 20; protein and acid compounds, 38; protein reaction, 43; pseudonucleins, 47, 151; lecithalbumins, 47, 349, 542; fat determination, 137; nucleins, 150, 151; mucosa of stomach, 349; yolk of egg, 502, 504-506, 510, 511; kidneys, 542; guaiac test, 648
v. Liebig, J., inosinic acid, 155; mineral bodies, 166; fat formation, 445; work and metabolism, 471, 472; urea, 557; diets, 764
Lieblein, V., wound-secretion, 265; liver and urea formation, 553
Liebrecht, A., 30
Liebreich, O., neurine, 145; protagon, 481; cholesterin fat, 691
Liepmann, W., 512
Lifschütz, J., isocholesterin, 337; wool-fat, 692
Likhatscheff, A., 637
Lilienfeld, L., histone, 62, 270; nucleohistone, 141, 270; cell nucleus, 142; fibrin ferment and blood coagulation, 176, 227; blood-plates, 222, 227; thymus, 271, 272; leuconuclein, 229, 270
v. Limbeck, R., 223
Limpricht, H., 455
Lindberger, W., tryptic digestion, 393; bile and putrefaction, 406
Lindemann, L., 111
v. Linden, M., 690
Lindvall, V., 73
Ling, A. R., 125
Lingle, D. J., 464
Linossier, G., 543
Linser, P., sebum, 691
Lintner, C. J., 128, 129
Lintwarew, S. J., pyloric reflex, 368; pancreatic juice, 383
Lipliawsky, A., 672
Lipp, A., 89
Lippich, Fr., 563
v. Lippmann, E. O., enzymes, 21; tyrosine, 90; carbohydrates, 105
Lister, J., 226
Lloyd-Jones, E., 222
Loeb, J., ion action, 166, 168, 169; muscles, 464; metabolism, 761
Loeb, L., blood coagulation, 232, 235

Loeb, W., carbon-dioxide assimilation, 1; muscle work, 474
Löbisch, W. F., bilipurpurin, 324; connective tissue, 428
Löbisch, W., mammary glands, 514; casein formation, 537
Löhlein, W., pepsin estimation, 357; trypsin estimation, 392
Lönnberg, J., cartilage, 433; kidneys, 542
Lönnquist, B., 351
Lörcher, G., 361
Loeschcke, K., 289
Loevenhart, A. S., enzymes, 7, 17, 446
Loew, O., active proteins, 4; catalases, 7, 20; protein nitrogen, 27; proteins, 30, 34, 49; sugar syntheses, 114; cell nucleus, 167
Loewenthal, W., 398
Loewi, O., phlorhizin diabetes, 298; sugar formation, 306; protein regeneration, 416; urea formation, 550; uric-acid formation, 571; allantoin, 584; conjugated glucuronic acids, 610; phosphorus metabolism, 619, 719
Löwit, M., 227
Loewy, A., diamines, 97, 98; alkalinity of the blood, 223; high altitudes, 246, 614, 762; work and metabolism with, 471, 760; amino acids in the urine, 614, oxygen absorption and pressure, 704, 705; metabolism, 762
Lohmann, lysine, 99; choline, 394; neurine, 615
Lohnstein, Th., urometer, 547; saccharometer, 657, 664; sugar estimation, 664
Lohrisch, H., 397
Lombroso, 421
London, E. S., digestion, 370, 400, 401; blood in starvation, 732
Long, J. H., casein, 518; urine coefficient, 627
Lorrain Smith, J., chlorose, 246; quantity of blood, 248
Lossen, F., 32
Lubarsky, E., 138
Luchsinger, B., glycogen, 291, 293; sweat, 693
Luciani, L., starvation, 244, 728
Ludwig, C., pseudohæmoglobin, 203; formation of bile, 330; gastric digestion, 369; pancreatic juice, 382; absorption of protein, 414; of sugar, 420; blood-gases, 696, 697; temperature and metabolism, 761
Ludwig, E., fat of demoid cysts, 138, 502; uric acid estimation, 576
Lücke, A., hyalin, 69, 687; pus, 269; benzoic acid reaction, 587
Lüdecke, K., glycerophosphoric acid, 144
Lüthje, H., sugar formation, 303–305; oxalic acid, 582
Lüttke, J., 376
Lukjanow, S., bile, 307, 308; starvation, 732
Lummert, W., fat, 283; fat formation, 445
Lunin, N., requirement for mineral bodies, 736; artificial feeding, 739
Lusk, Graham, phlorhizen diabetes, 298, 462; sugar formation, 306; lactose in the intestine, 419; lactic-acid formation, 462; fermentation of sugar, 665
Lussana, F., 295
Luther, E., 659
Luzzatto, A., 583

Maas, O., 49
Macullum, A. B., potassium, occurrence of, 167, 464; iron preparations, 245
Maccadam, J., 471
Mac Callum, J. B., 378
Macfayden, A., fermentation, 10; intestinal contents, 399
v. Mach, W., 572
Mackay, J. C. H., 652
Macleod, J., phosphocarnic acid, 457, 470; carbamic acid, 563
Mac Munn, Ch. A., hæmatoporphyrin, 213, 649, 690; urobilinoids, 214, 603; echinochron, 219; cholohæmatin, 324; myohæmatin, 454; tetronerythyrin, 690
Madsen, Th., 337
Maetzke, G., 400
Magnanini, G., 403
Magnier, 626
Magnus, G., 696
Magnus, R., 284
Magnus-Levy, A., thyreoidea, 277; liver, 281; diabetes, 306; fat formation, 445; hippuric acid, 586; volatile fatty acids in the urine, 608; Bence-Jones's proteid, 645; acetone bodies, 669, 674; respiration experiments, 713, 722, 723; metabolism, 757, 758, 762
Maillard, L. C., 594
Maillard, M. L., 168
Majert, W., 496
Makris, C., 530
Malcolm, J., 619
Malengreau, F., thymus and nucleohistone, 270, 271
Malfatti, H., tryptophan, 54; purine bases, 581
Mall, F., 80
Mallèvre, A., 397
Maly, R., oxyprotic acids, 31; peptones, 60, 746; bile-pigments, 320–323; hydrobilirubin, 320, 603; saliva, 341; secretion of hydrochloric acid, 364; pancreatic juice, 387; bile and putrefaction, 406; luteines, 505; creatinine, 566
Manasse, A. 92
Manasse, P., 283
Manché, M., 648
Manchot, W., oxidations, 6, 7, 19
Mandel, A. R., lactic acid formation, 462
Mandel, J. A., nucleic acids, 152, 153, 154, 514; spleen nucleoproteid, 272; glucothionic acids, 222, 272, 273, 285, 514; mammary gland, 514; organic phosphorus in the urine, 614

Mandelstamm, E., 308
Manicardi, C., 457
Manning, T. D., 378
Mansfield, G., 264
Maquenne, L., starch, 126; inosite, 458
Marcet, xanthine, 159; excretin, 410; excretolic acid, 410
Marchetti, G., 442
Marchlewski, L., pigments of leaves and blood, 197, 214; hæmin, 212; hæmopyrrol, 214; phylloerythrin and cholohæmatin, 324
Marcus, E., serglobulins, 178
Marcuse, G., phosphorus metabolism, 719
Marcuse, W., glycogen, 468; lactic acid formation, 469
Mares, Fr., 569
Marfori, P., 630
Margulies, 658
Mark, H., 283
Marquardsen, E., 407
Marshall, J., 597
Martin, C. J., fibrin formation, 176; blood coagulation, 234
Martin, S. H., 397
Martius, F., 376
Martz, 692
Maschke, O., protein crystals, 38; creatinine, 565
Masius, J. B., stercobilin, 320, 409
Masloff, A., 377
Massen, V., 552
Masuyama, M., 503
Mathieu, E., 697
Mathews, A., arbacine, 62; protamines, 63, 498; lysine, 98; nucleic acids, 154, 498; ion action, 169; fibrinogen, 172
Matthes, M., intestinal contents, 407; urinary pepsin, 615; urinary histone, 647
Maurenbrecher, A. D., 113
Mauthner, J., cholesterin, 334; asparagin, 1; nutritive value of, 747
Maximowitsch, S., seralbumin, 181, 182
May, R., 111
Mayer, A., 626
Mayer, J., 753
Mayer, L., 418
Mayer, M., 188
Mayer, P., cystine, 92-94; mannoses, 108, 109; conjugated glucuronic acids, 122, 123, 184, 609, 610, 667; oxalic acid, 583; indican, 593; skatoxyl glucuronic acid, 595
Mayo-Robson, A. W., 307
Mays, K., trypsin, 390; fuscin, 491
Mazé, P., 21
Meara, F. S., 52
Medwedew, A., oxidations, 818; glycocholic acid, 312
v. Mehring, J., urochloralic acid, 122, 632; blood-sugar, 184; portal blood, 242, 419; glycogen formation, 291; phlorhizin diabetes, 297, 298; pancreas diabetes, 300; amylolysis, 344, 388; assimilation of proteins, 412; absorption of sugar, 420; sarcosin, 631; acetonuria, 670
Méhu, C., pleural fluid, 261; urobilin, 604, 605
Meillère, G., 616
Meinert, C. A., utilization of foodstuffs, 418; diets, 764
Meinertz, J., 283
Meisenheimer, J., fermentation, 11, 21
Meisl, E., 445
Meissl, Th., 513
Meissner, G., products of digestion, 359; egg-white, 506; urea formation, 553; allantoin, 583; hippuric acid, 585
Meister, V., 553
Mendel, L. B., lymph formation, 255; alcohol, 369; trypsinogen, 385; absorption of proteid, 412, 413, 414; muscle extractives, 455; uric acid, 574; allantoin, 584; kynurenic acid, 600
Mendes de Leon, M. A., 531
Menzies, J. A., 205
De Merejkowski, C., 690
Mesernitzki, 502
Messinger, J., 673
Mester, Br., intestinal putrefaction, 407; indol and skatol, 596; urinary sulphur, 611
Mett, S., estimation of pepsin, 357; estimation of trypsin, 392
Meyer, C., 286
Meyer, E., blood-pigment and bile, 329; alcaptonuria, 598, 600; nitrobenzene, 635
Meyer, G., 417
Meyer, H., camphor glucuronic acid, 122; adrenaline substances, 278; uric-acid formation, 572
de Meyer, J., 302
Michaelis, H., 185
Michaelis, L., 186
Michel, A., seralbumin, 181-183
Micko, K., excrements, 409; absorption of casein, 719
v. Middendorff, M., 242
Miescher, F., protamines, 63, 64, 497; nucleins, 149, 152; pus, 267, 269; sperm, 497; salmon, metabolism of, 738
Milchner, R., 67
Millon, M. E., protein reaction, 30, 43; lactoprotein, 523
Mills, W., 582
Milroy, J. A., 209
Milroy, T. H., nucleins, 150; phosphorus metabolism, 619
Minkowski, O., blood alkalinity, 223; ascites, 262; glycogen, 295; blood-sugar and liver, 297; phlorhizin diabetes, 297, 298; pancreas diabetes, 300, 301; formation of bile pigments, 331-333; fat absorption, 421; pancreas and absorption, 418, 425; lactic acid, 460, 572, 573; uric acid 572, 573; allantoin,

584; histozyme, 588; acetone bodies, 670, 673, 674; blood in diabetes, 702
Mitjukoff, K., 500
Mittelbach, F., fibrinogen, 173; homogentisic acid, 600; estimation of protein, 646
Miura, K., blood-sugar, 184; glycogen formation, 290; intestinal invertase, 379
Miyamota, S., 355
Mochizuki, J., 394
Modrzejewski, E., 70
Möllenberg, R., 243
Möller, J., 409
Mörner, C. Th., membranins, 69, 434; albumoid, 74; gelatine, 77, 79, 80. 433, 434; ichthylepidin, 81; tyrosin test, 91; vitreous humor, 429, 491; cartilagenous tissue, 430–433, 435; cornea, 434, 492; bones, 436; crystalline lens, 492, 493; ovomucoid, 508; perkaglobulin, 510; homogentisic acid, 600; estimation of chlorine, 619; gallic and tannic acids, 637
Mörner, K. A. H., sulphur of the proteins, 28; cysteine and cystine, 28, 74, 92; thiolactic acid, 28, 74, 94; hydrolysis of protein, 29; albuminates, 49, 45; tyrosin, 89; proteids of the serum, 170–182; hæmatin and hæmin, 212, 213; blister fluid, 265; estimation of hydrochloric acid, 576; chondroitin sulphuric acid, 431, 542, 647; pigments of muscles, 454; urinary nitrogen, 548, 554; estimation of urea, 561; fatty acids in the urine, 608; nubecula, 615; acetanilide, 634; proteid in the urine, 640; nucleoalbumin in the urine, 646, 647; melanins, 651, 688; bile-acids in the urine, 652.
Mohr, Fr., reagent for hydrochloric acid, 374; chlorine titration, 617
Mohr, L., sugar formation, 306; urinary purines, 579; oxalic acid, 583
Mohr, P., 73
Moitessier, J., blood catalases, 20; carbon monoxide methæmoglobin, 207; chlorine compounds in the urine, 616; Bence-Jones proteid, 645
Moleschott, J., 764
Molisch, H., 117
Moll, L., 46
Monari, A., creatinine, 455, 470; muscle-work, 468, 470; xanthocreatinine, 567
Monéry, A., 431
Montuori, A., 297
Moor, Ovid, 562
Moore, B., bile and fatty acids, 398, 422; reaction of intestine, 407; synthesis of fat, 421; fat emulsion, 422
Moore, J., 115
Moraczewski, W., formation of fæces, 409; digestion of casein, 521; diabetes, 593
Morat, J., work and carbohydrates, 468
Morawitz, P., blood coagulation, 227, 231, 232, 234; cadaver-blood, 235; detection of proteoses, 644
Morax, V., 589
Moreau, A., 711
Moreau, J., 129
Morel, A., fibrinogen, 172, 173; blood lipase, 185; hæmatogen, 503
Morgen, A., 536
Morgenroth, J., antichymosin, 25, 361
Mori, Y., foodstuffs, utilization of, 418; diets, 764
Moriggia, A., 693
Morishima, K., liver, 281; lactic acid, 461
Moritz, 366
Moritz, F., transudates, 258; phlorhizin diabetes, 298; alimentary glycosuria, 298
Moriya, G., lactic acid, 460
Morkowin, N., 63
Morochowetz, L., 430
Morris, G. H., fermentation, 10; isomaltose, 125; amylolysis, 128
Moscatelli, R., ascites, 259, 263; lactic acid, 469, 608
Mosen, R., 222
Mosse, M., pseudochylose fluid, 262; blood sugar, 296; acid formation in the stomach, 364; ethereal sulphuric acids, 589
Mott, F. W., cerebrospinal fluid, 264, 488; diseases of the nervous system, 488
Mouneyrat, A., 102
Mühle, P., 57
Mühsam, J., 241
Müller, Erich, digestion of cellulose, 397
Müller, Ernst, bile-acids, 312, 313, 316, 318
Müller, Franz, high altitudes, 246, 762
Müller, Friedrich, autolysis of pneumonic infiltrations, 23, 268, 713; glucosamine from protein substances, 32, 66–68, 500; mucous-membrane mucin, 66; starvation (indican), 404; fat absorption, 424, 425; ethereal sulphuric acids, 589; urobilin, 603, 607; urinary sulphur, 611, 612; demolition of aniline, 633; acetone bodies, 668; fæces nitrogen, 718
Müller, Johannes, work and sugar consumption, 469; enzymes of the egg-yolk, 502
Müller, Julius, 591
Müller, M., 457
Müller, Paul, proteoses, 60; koprosterin, 337; excrements, 409; utilization of casein, 530, 719
Müller, Paul Th., fibrinogen, 172, 188; bone-marrow, 172, 437
Müller, W., cerebrin, 483, 485
Müntz, A., 539
Münzer, E., formation of urea, 551; liver and urinary nitrogen, 554
Müther, A., 113
Muirhead, A., 552
Mulder, G. J., 73
Munk, H., 276
Munk, I., chyle and lymph, 251–253;

sulphocyanides, 343, 611; contents of the intestine, 407; absorption of protein, 414; of sugar, 420; of fat, 423, 424, 427; fat synthesis and fat formation, 421, 442, 445; smooth muscles, 478; milk, 525; formation of urea, 551; elimination of phenol, 589; work and catabolism, 471, 620; reaction for bile-pigments, 653; titration of sugar, 661; starvation, 730, 731; gelatine, nutritive value of, 745; nutritive value of proteoses, 746; of asparagin, 747; protein requirement, 750; water and metabolism 753
Murray, Fr. W., 387
Musculus, F., amylolysis, 128, 344, 388; urochloralic acid, 632; urease, 677
Mygge, J., 646
Mylius, F., iodide of starch, 127; iodocholalic acid, 316; bile acids, 311, 312, 315–317

v. Naegeli, C., 126
Naegeli, O., 545
Nagano, J., intestinal juice, 378; absorption, 419, 420
Nagel, W., 697
Nakaseko, R., 290
Nakayama, M., erepsin, 153, 380; reaction for bile-pigments, 653
v. Name, W. G., gelatine, 77, 80
Nasse, H., blood, 243; lymph, 254; spleen, 273
Nasse, O., activation of oxygen, 5; protein bodies, 27, 42, 79; dextrines, 129; glycolysis, 185; glycogen, 287, 289, 467, 468; saliva, 344, 346; musculin, 450, 452; smooth muscles, 477
Naunyn, B., formation of glycogen, 291; liver and bile, 331–333; demolition of aromatic substances, 633
Nawratzki, 264
Nebelthau, E., glycogen, 291; hæmatoporphyrin, 650
Neilson, H., 342
Neimann, W., conjugated glucuronic acids, 122, 123, 609, 610; carbohydrate-like substance of the liver, 285, 296
Nencki, L., 635
v. Nencki, M., oxidation, 8; protein sulphur, 28; putrefaction of proteins, 30; trytophane, 102; blood-pigments, 197, 201, 211–213; phyllocyanin, 214; urobilinoids, 603; hæmatoporphyrin, 213, 214, 332; ammonia, 241, 550, 624, 625, 745; diabetes, 300; gastric enzymes, 354, 355; gastric juice, 354, 364; splitting of esters, 389; intestinal digestion, 398, 399, 401; indol, 402; reaction of the intestine, 407; butyric acid fermentation, 445; urea, 455, 549, 551, 553; carbamic acid, 552; urorosein, 601, 651; acid amides, 630; demolition of aromatic substances, 634, 635, 637; urine, odor of, 639; melanins, 688
Nerking, J., determination of fat, 137; glycogen, 289
Nernst, W., permeability of a membrane, 142; fluid chains, 192
Nessler, 374
Neubauer, C., creatine, 456; creatinine, 563, 566, 567; ammonia, 624
Neubaner, O., protein reaction, 43; alcaptonuria, 598; urobilinogen, 605; conjugation of glucuronic acid, 632; Ehrlich's reaction, 675
Neuberg, C., acetone, 32, 668; glucosamine, 67, 121, 500, 504; amyloid, 69, 70; amino-acids, isolation of, 92; cystine, 92–94; isoserine, 96; oxyaminosuccinic acid, 96; tetraoxyaminocaproic acid, 96, 431; diamines, 97, 98; mannoses, 108; pentoses, 109, 111–113; osazones, 117; levulose, 118, 119, 259; glucuronic acid, 121–123; conjugated glucuronic acids, 122, 123, 609, 610; lysine in the blood, 186; liver, 284; glycogen, 290; deamidation, 305; cholesterin, 335, 336; chondrosin, 431; galactosazone, 524; heteroxanthine, 579; estimation of phenol, 591; skatoxyl glucuronic acid, 595; mineral metabolism, 616, 626, 719, 737; phenylhydrazine test, 658; detection of glucuronic acids, 667
Neumann, Alb., orcin test, 112; nucleic acids, 152–155; pyrimidine bases, 152, 165; urinary iron, 626; phenylhydrazine test, 658
Neumann, E., 332
Neumann, O., nutritive value of alcohol, 755; protein requirement, 766
Neumann, R., 754
Neumann, Walt., 57
Neumeister, R., proteoses and peptones, 51, 52, keratins, 73; tryptophane, 102; dextrins, 129: glycogen, 289; absorption of proteins, 412, 413; ovomucoid, 508
Neusser, E., 649
Nicklés, J., 509
Niemilowicz, L., purine bases, 582; reducing power of the urine, 609
Nierenstein, E., 357
Nilson, G., 127
Nilson, L. F., milk-fat, 527, 528
Le Nobel, C., urobilinoids, 214, 603; hæmatoporphyrin, 649; acetone test, 67
Noeggerath, C. T., 739
Noel-Paton, D., lymph, 253; liver, 282; formation of fat, 294, 296; bile, 307, 328; work and protein, 471
Noguchi, H., 337
Nolf, P., fibrinogen, 172, 175; fibrinolysis, 175; proteoses in the blood, 183, 415; blood coagulation, 234; saliva, 340; carbamic acid, 563
Noll, A., nucleic acid, 154; protagon, 488
v. Noorden, C., spectrophotometry, 218;

alimentary glycosuria, 298; diabetes, 301; formation of sugar, 306; liver and urinary nitrogen, 554; ethereal sulphuric acids, 589; proteid in the urine, 640; metabolism, 733, 750, 752, 753
Notkin, J., 276
Nothwang, Fr., 734
Novi, J., pseudohæmoglobin, 203; iron of the liver, 285; of the bile, 325; saliva, 347
Novy, F., 49
Nowak, J., meat, 476; respiration apparatus, 712; nitrogen deficeti, 718
Nussbaum, M., alveolar air, 708; carbondioxide tension, 709
Nuttal, G., 405
Nylander, E., sugar test, 116, 655
Nylén, S., 345

Obermayer, Fr., precipitation of protein, 45; indican test, 594
Obermüller, K., saponification, 136; cholesterin, 334, 335, 336
Oddi, R., bile in the stomach, 398; amyloid, 431; acidity of the urine, 544
Odenius, R., 514
Oertel, diet cures, 770, 771
Oertel, Horst, phosphorus compounds in the urine, 614; phosphorus metabolism, 719
Oertmann, E., oxidations, 3, 723
Oerum, H. P. jr., blood-pigments, 217; human bile, 327; estimation of indican, 595
Oerum, H. P., cystic fluids, 502; nutritive value of gelatine, 745
Offer, Th. R., glycogen, 294; nutritive value of alcohol, 755
Ofner, R. 119
Ogata, M., 369
Ogden, H., 598
Oidtmann, H., lymph-glands, 269; thymus, 272; spleen, 274; thyroid, 275; salivary gland, 339; pancreas, 382; kidneys, 542; lungs, 714
Oker-Blom, M., dissociation, 190; suprarenal capsule, 277
Okunew, W., 56
Oliver, G., 278
Ollendorff, G., 107
Olsavszky, V., 620
Omeliansky, 23, 397
van Oondt, 298
Oppenheim, M., 20
Oppenheimer, C., ferments, 12, 13, 390; seralbumin, 181; proteoses in the blood, 183; parental protein assimilation, 412; law of surface, 757
Orbán, R., 379
Orgler, A., acetone, 32, 668; tetraoxyaminocaproic acid, 96, 431; uric acid and nutrition, 569
Orglmeister, G., 97
Orndorff, W. R., bilirubin, 320, 321
Orton, K. J. P., 599, 600
Osborne, T. B., proteins, 27, 28, 46, 62; nucleic acids, 152, 156; pyrimidine bases, 152, 156; ovovitellin, 503; ovomucin, 506; ovalbumins, 507
Osborne, W. A., 459
Ost, H., 125
Ostwald, W., catalysis, 14; action of ions, 168
Oswald, A., halogen proteins, 31; thyroid gland, 275–277; urinary globulins, 643
Otori, J., mucin, 67; transudates, 259; autodigestion of the pancreas, 394; pseudomucin, 501
v. Ott, 415
Ott, A., transudates, 259; phosphates, 619
Otto, J. G., blood-sugar, 184, 237; blood, 242, 243, 419; blood-plasma, quantity, 238; blood-pigments, 197, 204, 205, 218; skatoxyl-glucuronic acid, 595; estimation of sugar, 660, 662
Overton, E., boundary layer of the protoplasm, 142, 143, 148; permeability of the blood corpuscles, 195; of the muscles, 465; mineral bodies of the muscles, 464
Owen-Rees, 251

Paal, C., proteins, 27, 49, 51, 53, 59; gelatine peptone, 79, 80; animo-acids, 102
Pachon, V., coagulation of the blood, 234, 235; extirpation of the stomach, 369, 371; trypsinogen, 385
Paderi, 185
Pagès, C., coagulation of the blood, 229, 230; rennet coagulation, 520; milk, 536
Paijkull, L., exudates, 257, 258, 261, 262; bile-mucus, 310
Painter, H. M., proteoses, 52; saliva, 346
Panek, K., oxyproteic acids in the urine, 612, 613; uroferric acid, 614; phosphaturia, 621
Panella, A., phosphoric acid, 186, 457, 477
Panormoff, A., muscle-sugar, 459; ovalbumin, 507; egg-white, 508
Pantanelli, E., 87
Panum, P. L., serum casein, 178; blood in starvation, 244, 732; transfusion, 245, 248; elimination of urea, 744; nutritive value of gelatine, 745
Panzer, Th., halogen protein, 32; chyle, 251; cerebrospinal fluid, 264; colloid, 499, 500
Paraschtschuk, S., 538
Parastschuk, S. W., 362
Parcus, E., cerebrin, 483; homocerebrin, 483
Parke, J. L., 505, 506
Parker, W. H., 422
Parmentier, E., 530
Partridge, C. L., purine-base enzymes, 158, 274, 571
Pascheles, W., 631
Paschutin, V., lymph, 254; intestinal juice, 378
Pascucci, O., blood-corpuscles, 193, 194
Pasqualis, G., 608

Pasteur, L., fermentation, 10, 11; micro-organisms and digestion, 405
Patten, A. J., cysteine, 28, 94; histidine, 100; hexone bases, 100
Paul, Th., action of poison, 168 uric acid, 575
Pauli, W., protein, 39, 40; gelatine solutions, 78
Pauly, H., histidine, 99; adrenaline, 278
Pautz, W., aqueous humor, 265; lactase, 379; vitreous humor, 491
Pavy, F. W., carbohydrate in proteins, 32, 293; isomaltose, 184; glycogen, 290, 295; blood sugar and diabetes, 295–298; autodigestion of stomach, 372; work and metabolism, 471; estimation of sugar, 660, 663
Pawlow, J. P., biliary fistula, 307; saliva, 342, 348; stomach and gastric juice, 349, 351, 353, 356; gastric enzymes, 354, 362; pylorus-reflex, 368; pancreatic juice, 382–384, 386; enterokinase, 383; pancreas enzymes, 386, 389, 392, 397; ammonia in the blood, 551; Fick's fistula, 552; urea formation, 553
Payer, A., 243
Peiper, G., 223
Péju, P., 173
Pekelharing, C. A., tryptophane, 54; fibrin ferment and blood coagulation, 175, 176, 229, 230; nucleoproteids, 178, 451; gastric enzymes, 355, 356, 362
Pemsel, W., 38
Penny, E., 589
Penzoldt, Fr., acetone, 671, 673
Pernossi, L., 12
Pernou, M., 274
Peskind, S., 193
Petersen, P., 475
Petit, A., 616
Petrone, A., 227
Petrowski, D., 486
Petry, E., blood-corpuscles, 196; rennet coagulation, 521
v. Pettenkofer, M., test for bile acids, 311; formation of fat, 442–444; work and metabolism, 471–473, 759; respiration apparatus, 712, 722; metabolism experiments, 716, 718, 741, 759; diets, 764
Pfaff, F., 307
Pfannenstiel, J., 499
Pfaundler, M., digestion products, 53, 55; nitrogen in the urine, 549
Pfeiffer, E., woman's milk, 531, 533
Pfeiffer, L., 688
Pfeiffer, Th., 587
Pfeiffer, W., 574
Pfleiderer, R., 359
Pflüger, E., oxidations, 3, 4, 723; ethereal sulphuric acids, 280; glycogen, 288-292, 434; diabetes, 301–303, 306; gases of the saliva, 340; bile and fatty acids, 397, 423; fat absorption, 422, 423; fat formation, 443, 445, 741; nitrogen in meat, 444; muscle metabolism, 467, 469, 471, 473, 474; ovaries, 498; gases of milk, 528; urinary nitrogen, 548; estimation of, 560, 561; estimation of urea, 557–561, 613; sugar tests, 657, 663, 664; blood-gases and respiration, 251, 696, 697, 700, 702, 708–711; respiration apparatus, 712; N : C quotient in the urine, 720; calorific value of oxygen, 723; protein metabolism, 739, 741, 742, 751; nutritive requirements, 742; external temperature and metabolism, 761; protein requirements, 765
Phisalix, C., 693
Picard, J., 491
Piccard, 63
Piccolo, G., hæmatoidin and lutein, 216, 498
Pick, A., 358
Pick, E. P., proteoses and peptones, 53, 55–57, 60; serglobulin, 178; peptozyme, 234
Pick, Friedel, 295, 296
Pickardt, M., transudates, 259; cartilage, 434
Pickering, J. W., products similar to the proteins, 34, 234
Piegand, J., 259
Pierallini, G., 630
Piettre, M., blood-pigments, 202, 212
Piloty, O., glucosamine, 121; conjugated glucuronic acids, 610
Pilzecker, A., 329
Pinkus, S. N., proteins, 30, 39; crystallization, 182, 507, 508
Piontkowski, L. F., 351
Piria, 90
Planer, J., 372
Plattner, E., 310
Plaut, M., 614
Playfair, 764
Plimmer, R. H., lactase, 386
Plósz, P., blood corpuscles, 194; liver, 281, 282; elimination of proteoses, 414; nutritive value of proteoses, 746; urinary pigments, 601; urinary protein, 640
Poda, H., excrements, 409; absorption of casein, 719
Poduschka, P., 574
Poehl, A., intestinal putrefaction, 405; spermine, 496
Pohl, J., oxidations, 8; dextrin, 129; estimation of globulin, 180, 646; liver, 281; urea formation, 551; allantoin, 574, 584; oxalic acid, 630; demolition of fatty bodies, 630
Poleck, egg, 505, 509
Polimanti, O., estimation of fat, 137; fatty degeneration, 443
Politis, G., 747
Pollak, L., glutinase, 391, 392; sulphocyanide, 631
Pollitzer, S., 746
Pommerehne, H., 563

Ponfick, E., 248
Ponomarew, Brunner's glands, 377
Popel, W., 244
Popielski, L., enterokinase, 383; pancreatic juice, 383, 384
Popoff, N., 415
Porcher, Ch., lactose, 539; urinary indican, 592–595; skatol red and urorosein, 596; uroerythyrin, 607
Porges, O., 179
Porteret, E., 291
Portier, P., fermentation, 21; lactose in the intestine, 419
Posner, C., semen, 495, 496; urinary protein, 640
Posner, E. R., mucin, 67, 68; mucoids, 360; protagon, 482
Posselt, L., 82
Posternak, S., musculamine, 457; oxymethylphosphoric acid, 458
Pottevin, H., isomaltose, 125; syntheses of esters, 390
Pouchet, A. G., carmine, 456; urinary purines, 578; urinary poison, 615; lungs, 714
Poulet, V., 713
Poulsson, E., 455
Pozerski, E., kinases, 383; secretin, 384
Pozzi-Escot, E., 19
Prausnitz, W., phlorhizin diabetes, 298; excrements, 409; absorption of casein, 719; metabolism in starvation, 729, 730
Predteschensky, E., 761
Pregl, Fr., protein hydrolysis, 88, 101; carbon monoxide hæmochromogen, 209; dehydrocholon, 311, 316; bile-acids, 316–318; intestinal juice, 377, 378; ovalbumin, 507; oxyproteic acid, 613; peptides in the urine, 614; C. : N. quotient, 628
Presch, W., urinary sulphur, 611; hyposulphrites, 612
Preusse, C., phenols in the urine, 591; behavior of aromatic substances, 633, 639
Prevost, J. L., bile, 426; formation of urea, 553
Preyer, W., globin, 208; blood-crystals, 208; placenta-pigment, 512
Preysz, K., 620
Pribram, E., 633
Pribram, R., 623
Pribram, H., 453
Prochownick, L., 513
Pröscher, F., bilirubin, 321; milk, 529, 536; Erhlich's urine test, 675
Prutz, W., 592
Prym, O., spleen and digestion, 274, 385; trypsin, 383
Pugliese, A., 232
Puls, J., 525
Pupkin, Z., 223

Quevenne, Th., lymph, 252; witch's milk, 534
Quincke, G., 517
Quincke, H., hæmatoidin, 216; iron preparations, 245; poikilozytosis, 247
Quinquaud, Ch., urea, 240, 242; muscle-work, 468; fatty acids in the urine, 629

Raaschou, C. A., guanylic acid, 155, 156
Rachford, B. K., pancreatic diastase, 388; trypsin digestion, 393; bile and fat, 398
Radenhausen, P., 517
Radziejewski, S., 442
Radzikowski, C., 350
Raehlmann, E., 491
Raikow, P. N., 28
Raineri, G., 512
Ramsden, W., 61
Ranke, H., 569
Ranke, J., division of blood, 249; bile, 307
Ransom, H., hæmolysis, 193, 337
Rapp, R., 10
Rauchwerger, D., cholesterin, 335, 336
Raudnitz, R. W., milk and casein, 515, 519, 521
Reach, F., tyrosine, 89; pseudopepsin, 355; digestion, 370; protein absorption, 413; muscular activity, 474
Reale, E., oxalic acid, 582; urinary sulphur, 611
Reese, H., 614
Regnault, H. V., cutaneous respiration, 695; method of respiration experiment, 712; nitrogen deficit, 722
Reh, A., 269
Reich, O., 625
Reichel, H., 521
Reich-Herzberge, F., 395
Reinbold, R., Molisch's reaction, 117; methæmoglobin, 204; trypsin digestion, 394; benzoylation of carbohydrates, 659
Reinders, W., 14
Reinecke, 441
Reiset, J., cutaneous respiration, 695; method of respiration experiment, 712, 722; nitrogen deficit, 718
Reiss, E., 180
Reissner, O., 376
Reitzenstein, A., 578
Rekowski, L., 637
Rennie, J., pancreas, 302, 381
Renwall, G., 625
Rettger, L. F., 385
Reuss, A., transudates, 259, 261
Reye, W., 174
Reynolds, J. E., 671
de Rey-Pailhade, J., enzymes, 8, 19
v. Rohrer, L., acid compounds of proteins, 38; acidity of the urine, 545
Riazantseff, N. V., elimination of nitrogen, 745, 762
Ribaut, H., enzyme actions, 17, 19
Richards, A. N., albuminoids, 75, 76, 77; hexone bases, 100; saliva, 343
Richet, Ch., gastric juice, 349, 353; digestion, 368; fat formation, 446,

urea, 550; uric acid, 574; thalassin, 693; method of respiration experiment, 713, 722; law of surface, 756
Richter, Max, 496
Richter, P. F., liver, 186, 284, 554
Riecke, R., 587
Rieder, H., 718
Riegel, M., 518
Riegel, secretion of gastric juice, 353
Riegler, R., 210
Riess, L., oxymandelic acid, 597; lactic acid, 608
Ringer, L., 229
Ringstedt, O. T., 544
Ritter, A., phlorhizen diabetes, 298; fat absorption, 42; fermentation of urine, 677
Ritter, E., bile, 328, 329; gall-stones, 334
Ritter, E., cholesterin, 338
Ritthausen, H., proteins, 29, 38; leucinimide, 87; milk, 525
Riva, A., urinary pigments, 601, 607, 650
Rivalta, F., 258
Roberts, W., pancreas rennet, 396; estimation of protein, 646; estimation of sugar, 663
Roch, G., 642
Rockwood, C. W., 614
Rockwood, D., bile and fatty acids, 398, 422; fat absorption, 422; reaction of the intestine, 407
Rockwood, E. W., protein assimilation and absorption, 413; uric acid, 570
Rodier, A., 243
Rödén, H., 361
Roeder, G., pyrimidine bases, 164
Röhmann, F., oxidation enzymes, 8, 18; leucin ethylester, 86; detection of glucose, 118; amylolysis, 125, 345; glycolysis 185; fat in the blood, 241; diastase, 185, 251, 295, 297; glycogen formation, 291; intestinal juice, 378; bile and putrefaction, 406; absorption, 419, 421, 424; muscles, 447, 466; casein salts, 519; phosphorus metabolism, 619, 719, 738; sebum, 691; anal-gland secretion, 692; artificial nutrition, 739
Röhrig, A., metabolism of the muscles, 467; cutaneous respiration, 695
Röse, B., 521
Rösing, E., 6
Roger, G. H., 280
Rohde, E., 43
Rokitansky, P., 608
Rona, P., histone, 62; gelatine, 78; α-proline, 101; sugar formation, 296; duodenal secretion, 377; protein regeneration, 417
Ronchi, J., cutaneous respiration, 695
Roos, E., iodothyrine, 276; phosphorus metabolism, 619; sugar tests, 658, 659
Roosen, O., 568
Rosa, E., 713
Rosemann, Rud, milk, 539; eliminatio of nitrogen, 744; nutritive value of alcohol, 755
Rosenbach, O, urine tests, 652, 675
Rosenbaum, A., blood catalases, 20; glycolysis, 303
Rosenberg, Br., 298
Rosenberg, S., secretion of bile, 308; pancreatic juice, 383; bile and putrefaction, 406; pancreas and absorption, 418, 421, 425
Rosenfeld, Fritz, indican, 593; volatile fatty acids, 608
Rosenfeld, G., fat and fat formation, 282, 283, 442, 443; glycogen and fat, 283; uric acid, 569; phenylhydrazine test, 658; acetone bodies, 669
Rosenfeld, M., 210
Rosenfeld, R., 184
Rosenheim, Th., 750
Rosenqvist, E., 306
Rosenstein, A., chyle and lymph absorption, 252, 253, 414, 420, 423
Rosenstein, W., 299
Rosenthal, J., 712
Rosin, H., levulose, 119, 665; indican, 594; skatol pigments, 596; reducting power of the urine, 609; Rosenbach's urine test, 675
Rossi, O., 264
Rost, E., gallic and tannic acids, 637; salts and metabolism, 754
Rostoski, O., 645
Roth, W., 256
Rothberger, C. J., liver, 280; carbamic acid, 552
Rothera, C. H., protein nitrogen, 27; cystine, 92
Rotmann, F., 259
Rotschy, A., 214
Roux, E., 126
Rovida, C. L., hyaline substance, 141, 194, 267
Rovighi, A., putrefaction, 405, 589
Rowland, S., fermentation, 10; leinases, 273; muscle enzymes, 454
Rubbrecht, R., 188
Rubner, M., protein sulphur, 28; sugar reaction, 117, 658, 665; utilization of foodstuffs, 417, 421, 424, 530, 727, 763; nitrogen in meat, 444; fat formation, 445; milk, 532; nitrogen of fæces, 718; heat of combustion of foodstuffs, 724–727; metabolism experiments, 716, 726, 730, 752, 756–758, 761; law of surface, 757, 758
Rubow, W., 464
Rüdel, G., 575
Ruff, O., 107
Ruge, E., 404
Rulot, H., fibrinolysis, 174, 175
Rumpf, Th., sugar formation, 306; estimation of phenol, 589; ammonia, 625
Runneberg, J. W., 261
Ruppel, W. G., protagon, 481, 482; milk-fat, 530; vernix caseosa, 691

Russel, 523
Russo, M., 687
Rywosch, D., glycolysis, 185; blood-corpuscles, 193

Saarbach, L., 204
Sabanejew, A., 60
Sabbatani, S., 229
Sacharjin, 238
Sacharow, N., 57
Sachs, Fritz, nucleases, 153, 380, 395; elimination of salts, 364
Sachsse, R., carbohydrate, 116, 127
Sackur, O., casein, 519, 520
Sadikoff, W., 77
Sahli, H., hæmometer, 219; titration of sugar, 663
Saiki, T., 608
Saillet, urobilin and urobilinogen, 601–606; hæmatoporphyrin, 213, 650
de Saint Martin, L., 205
Saint Pierre, C., 711
Saito, S., lactic acid, 241, 461
Salaskin, S., digestion products, 53; plastein, 56; leucinimide, 87; alkalinity of the blood, 223; ammonia, 241, 551, 624, 745; erepsin, 380; urea, 550, 553, 561; liver and acid formation, 554, 572; uric acid formation, 572
Salkowski, E., oxidation enzymes, 8; autodigestion, 22; putrefactive products, 30, 401; protein, 40, 43; pseudonucleic acid, 46, 521, 522; pseudonuclein, 47; proteoses, 52, 644; skatolcarbonic acid, 102, 596; pentoses, 110–112, 290, 666; glucuronic acid, 121; cerebrospinal fluid, 264; synovin, 265; liver proteid 282; glycogen, 290; cholesterin, 336; saliva, 347; pancreas, 391; indol, 403; adipocere, 443; nitrogen of meat, 476; ovomucoid, 508; casein, 521, 522; urea, 550, 551, 553; creatinine, 565, 566; uric acid, 573, 576; purine bases, 581; oxalic acid, 582, 583, 630; allantoin, 584; hippuric acid, 585; phenaceturic acid, 588; ethereal sulphuric acid, 589; indican, 595; urobilin, 605, 644; urine, fatty acids of, 608, 677; carbohydrates of, 609; sulphur compounds of, 611, 612; alkalies of, 623; estimation of the sulphur acids of the urine, 623; destruction of various substances, 631, 632, 634, 636; hæmatoporphyrin, 649, 650; fermentation test, 657; estimation of acetone, 673; water and metabolism, 753
Salkowski, H., putrefaction, 401, 585; demolition of aromatic substances, 634
Salomon, George, glycogen, 148; purine bases, 158; lactic acid, 241; urine purines, 578–580
Salomon, H., dieamidation, 305; oxalic acid, 583; acetone, 670
Salomon, W., 551
Salvioli, G., 414, 415
Sammis, J. L., 767
Samuely, F., melanoidins, 30, 689; amino-acids in the urine, 614; cystine, demolition of, 631
Sanders-Ezn, H., 761
Sandmeyer, W., pancreas diabetes, 301; absorption, 418, 425, 426; phosphorus metabolism, 719
Satta, G., urinary nitrogen, 549; acetone bodies, 669
Sauerbeck, E., pancreas, 302, 381
Sawitsch, W., 385
Sawjalow, W., plastein, 56; chymosin, 362
Schäfer, E., coagulation of blood, 229; suprarenal capsule, 287
Schaffer, Ph., uric acid, 578; ammonia, 625
Schaffer, F., 28
Schalfejeff, M., hæmin, 211, 212
Schardinger, F., 460
Scheermesser, W., peptons, 57, 58, 80
Scheibe, A., 532
Scheibler, C., 124
Schemiakine, A. J., 365
Schenck, Fr., detection of glucose, 118; blood-sugar, 240, 297, 469
Schenck, M., protein substances, oxidation of, 32, 582; martamic acid, 154
Schepowalnikow, N. P., 383
Schepski, N. W., 745
Scherer, J., lymph, 252; inosite, 458, 459, meta- and paralbumin, 500
Scheuer, M., 353
Scheunert, A., 367
Schierbeck, N. P., saliva, 345; gases of the stomach, 372; trypsin digestion, 393; fæces, 408
Schiff, A., 357
Schiff, H., protein, 27; biuret reaction, 43; cholesterin, 336; urea, 555; uric acid, 576
Schiff, M., spleen, 274; liver, 280; liver sugar, 295; bile, 309, 426; charging theory, 364, 385
Schindler, S., 271
Schittenhelm, A., xanthine oxidases, 18, 158, 273, 571, 574; elastin, 75; amino-acids, 83–85, 91, 614, 630; α-proline, 101; nucleic acids, 153; purine bases, 158, 163, 408; coagulation of the blood, 231, 233; spleen, 273; uric acid, 275, 571, 574; estimation of ammonia, 625; cystinuria, 675
Schlatter, K., digestion, 370, 371
Schlesinger, A., 345
Schlesinger, W., 606
Schlösing, Th., 625
Schlossberger, J. E., milk, 534, 539
Schlossmann, A., milk, 526, 529, 532
Schmey, M., liver, 285; muscle, 464, 477
Schmid, Jul., 581
Schmidt, Ad., intestinal putrefaction, 400; excrements, 410
Schmidt, Albr., 497
Schmidt, Alex., catalysis, 19; cell protein, 141, 228, 271; coagulation of the blood,

176, 177, 221, 227–232; fibrinoplastic substance, 178, 221, 228; blood corpuscles of the frog, 195; leucocytes, 221. 227–229; blood-gases, 698.
Schmidt, C., serum, 188; blood, 238, 239; lymph, 262; transudates, 257; buccal mucus, 341; saliva, 346; gastric juice, 353, 354; pancreatic juice, 387; biliary fistula, 406; absorption of fat, 424; osteomalacia, 439
Schmidt, C. H. L., 31
Schmidt, C. W., 714
Schmidt, E., 455
Schmidt, Fr., 670
Schmidt, P., 579
Schmidt-Mülheim, A., coagulation of the blood, 171; protein absorption, 413, 414, 744
Schmidt-Nielsen, S., enzymes, 12; chymosin, 362
Schmiedeberg, O., oxidations, 7; protein crystals, 38; desamino-albuminic acid, 49; salmine, 63; onuphin, 69; camphoglucuronic acid, 122, 638; nucleic acids, 152, 154, 155; nucleosin, 165; ferratin, 282; chondroitin sulphuric acid, 431, 432; urea, 551; hippuric acid, 586, 587; histozym, 588; phenolglucuronic acid, 590; indoxylglucuronic acid, 595; chitin, 686; melanin substances, 688
Schmitz, K., putrefaction, 405, 407, 589
Schneider, A., 238
Schneider, E., saliva, 343; kynurenic acid, 600
Schneider, H., tyrosinases, 18; melanins, 690
Schöffer, A., 85
Schönbein, C. F., 623
Schöndorff, B., urea, 240, 455, 532; estimation of, 562; thyroidea, 277; glycogen, 288, 292, 459; uric acid, 569; protein metabolism, 742, 743
Schöne, A., 111
Scholz, H., indican, 593
Shore, L. E., absorption, 415, 416
Schottelius, M., 405
Schotten, C., fellic acid, 318; intestinal putrefaction, 586; fatty acids in the urine, 608, 629; damaturic and damolic acids, 616; behavior of aromatic substances, 633, 634
Schoubenko, G., 28
Schoumow-Simanowski, E. O., pepsin, 355; gastric juice, 364, 371
Schreiber, E., 571
Schreiner, Ph., 496
Schreuer, M., nitrogen of meat, 477; calorific value of nitrogen, 727; protein overfeeding, 744, 752
Schrodt, M., 437
v. Schroeder, W., uric acid, 240, 241, 547, 572; urea, 551, 553
Schröter, F., 153
Schrötter, H , proteoses and peptone, 52, 53, 60
Schryver, S. B., 23
Schüle, 353
Schüle, A., 343
Schütz., E., digestion products, 55; estimation of pepsin, 357, 358; stomach, 366; lactic acid, 608
Schütz, J., pepsin, estimation of, 357; action of, 359; bile and fat, 398
Schütze, Alb., 186
Schützenberger, P., proteins, 29, 34, 51, 59
Schultze, B., 445
Schultze, E., 548
Schultzen, O., diabetes, 300; urea, 550, 551; oxymandelic acid, 597; lactic acid, 608; acid amides, 631; sarcosin, 631; behavior of aromatic substances, 633, 635
Schulz, Arth., 215
Schulz, Fr. N., protein, 28, 31, 33, 38, 41, 59; oxyprotein, 31; histones, 62, 208; galactosamine, 65, 71, 121; estimation of fat, 137; seralbumin, 181; starvation, 244, 730
Schulz, H., 429
Schulze, C., 345
Schulze, E., hydrolytic products of proteins, 29, 84, 85, 87, 90, 91; hexone bases, 96, 100; hemicelluloses, 130; isocholesterin, 337; lecithans, 144
Schulze, E., 522
Schulze, F. E., 166
Schumburg, W., chymosin, 361; metabolism, 758
Schumm, O., blood, 217, 247; spleen, 273; sugar formation, 306; pancreatic cysts, 387
Schunck, C. A., pigments, 197, 505
Schunck, E., 601
Schur, H., uric acid, 569, 570, 574; urinary purines, 579
Schurig, 285
Schuster, A., utilization of foodstuffs, 418; diets, 770
Schuurmanns-Stekhoven, 202
Schwalbe, E., blood plates, 227; fat, 283, 443
Schwann, Th., biliary fistula, 307, 406
Schwarz, H., 117
Schwarz, Hugo, elastin, 75, 76, 89
Schwarz, L., protein compounds, 49; secretion of hydrochloric acid, 364; acetone bodies, 669
Schwarz, O., antipepsin, 373; aceto-acetic acid, 672
Schwarzschild, M., biuret bases, 35; trypsin, 390, 395
Schweissinger, O., 642
Schwinge, W., 243
Scofield, H., 323
Sebelein, J., peptones, 52; digestion of casein, 521; milk, 515, 521, 522, 525, 528
Seegen, J., blood-sugar, 118, 184, 295, 296, 468, 473; carbohydrate substance in the liver, 285, 296, 297; amylolysis, 344;

respiration, 712; nitrogen deficit, 718; metabolism and water, 753
Seelig, P., 405
Seemann, J., proteins, oxidation of, 32, 78; carbohydrate in the proteins, 33, 508; nucleic acid, 154; erepsin, 380; intestinal contents, 400; absorption, 413; ovomucoid, 508
Segale, M., 166
Seitz, W., 287
Selitrenny, L., 78
Seliwanoff, Th., levulose, 119, 665
Selmi, 24
Semmer, G., 195
Senator, H., 593
Senkowski, M., bile-acids, 316
Senter, G., 20
Serdjukow, A., 368
Sertoli, E., 701
Sestini, L., 568
Setschenow, J., blood-gases, 697, 699, 701
Shepard, C. U., 585
Shore, L. E., protein absorption, 415, 416
Siau, R. L., isomaltose, 184; phlorhizin diabetes, 298
Sieber, N., protein sulphur, 28; glycolysis, 185; blood-pigments, 201, 211, 213; hæmatoporphyrin, 213, 214, 215, 332; urobilinoids, 214, 603; diabetes, 300; gastric juice, 354, 355, 362, 371; intestinal digestion, 399; Umikoff's reaction, 532; urorosein, 601, 651; nitrobenzaldehyde, 636; melanins, 688, 689
Siegert, F., 441
Siegfried, M., hydrolysis of proteins, 29; peptone substances and kyrines, 54, 58, 59, 60, 80; reticulin, 80, 81, 428; glutamic acid, 88; nitrotoluene sulpho-compounds, 92; lysine, 99; pseudohæmoglobin, 203; jecorin, 283; phosphocarnic acid, 457, 458, 462, 470, 474; orylic acid, 523; milk nucleon, 532; carbanimo-acids, 701
Silbermann, M., isoserine, 96; oxyanimosuccinic acid, 96
Silbermann, O., 333
Šimaček, E., fermentation, 21, 396; lactic acid, 461
Simon, G., milk, 526, 528
Simon, O., autolysis, 23, 268, 714; carbohydrate substance of the liver, 296
de Sinety, L., 665
Sivén, V. O., uric acid, 569, 570; protein metabolism, 738, 750, 765
Siwertzow, D., 145
Sjöqvist, J., proteins, 38, 60; estimation of hydrochloric acid, 375, 376; urinary nitrogen, 546, 554; urea estimations, 561
Skita, A., fibroin hydrolysis, 82–84, 89
Skraup, Zd., protein hydrolysis, 29, 78; oxyanimo acids, 96, 101; caseanic and caseinic acids, 101; benzoylation of carbohydrates, 117; kyrines, 58
Slosse, A., urinary nitrogen, 553, 744
Slowtzoff, B., pentosanes, 111; liver, 286; semen, 495; metabolism, 57, 759
Smale, Fr., 575
Smirnow, A., 636
Smith, F., 693
Smith, Herbert, saliva, 345, 346; bones, 435
Smith, Lowain, chlorosis, 246; quantity of blood, 248; oxygen tension, 707, 708
Smith, W. G., 654
Smith, W. J., urinary sulphur, 612; demolition of sulphur compounds, 631
Smits, H., 46
Socin, C. A., 293
Socoloff, N. 326
Söldner, F., milk, 518–520, 525–527, 531, 533–535
Sörensen, S. P. S., amino-acids, 83; ornithin, 97; α-proline, 102
Soetber, F., 621
Solera, L., 343
Solley, Fr., gelatine, 77, 79
Sollmann, T., gall-bladder, 329; muscles, 449, 453; fibroma of uterus, 502
Sommer, A., 283
Sommerfeld, 327
Sondén, K., respiration aparatus, 713, 722; metabolism, 756, 758, 768
Sorby, H. C., 509
Sourdat, 534
Southgate, 393
Soxhlet, F., glucose, 117; maltose, 125; fat formation, 445; milk, 518, 520, 526, 536, 538; titration of sugar, 661
Spampani, G., 538
Spangaro, S., 170
Speck, C., gas exchange, 713, 759–762
Spiegler, A., 734
Spiegler, E., protein reagent, 642; melanins, 689
Spiro, K., proteins, 38, 45; phenylalanine, 78; glycocoll, 83; serglobulin, 179; blood coagulation, 231, 232, 234; peptozym, 234; lactic acid, 469; rennet coagulation, 520, 521
Spitzer, W., oxidation enzymes, 8, 18, 20; xanthin oxidases, 18; glycolysis, 20, 185, 302; liver protein, 282; uric acid formation, 571
Spriggs, E. J., 357
Ssubotin, M., 536
Staal, J. Ph., 596
Stade, W., 363
Stadelmann, E., tryptophane, 102; icterus, 216, 332, 333; suprarenal capsule, 277, 330; bile, 307–309, 328, 331–333, 426; intestinal putrefaction, 407; elimination of nitrogen, 554; elimination of ammonia, 624; peptonuria, 643; β-oxybutyric acid, 673; blood in diabetes, 702
Stadthagen, M., diamines, 24, 615; adenine, 162; xanthocreatinine, 567; urinary sulphur, 611; poison of urine, 615; cystine, 676
Staedeler, G., 324

Staehelin, R., 258
Stanek, V., 145
Stange, M., 132
Starke, J., proteins, 40, 182; globulin, 46
Starke, K., 181, 182
Starling, E. H., lymph formation, 255, 256; intestinal enzymes, 379; enterokinase and trypsinogen, 379, 383–386; secretin, 384; pancreatic erepsin, 391
Stassano, H., 386
Stavenhagen, A., 10
Steensma, F. A., 402
Steiff, R., 589
Steiger, E., 96
Steil, H., muscle-fat, 474, 475
Stein, G., 334
Steinitz, phosphorus metabolism, 619, 719; C.: N. quotient, 628, 720; lactose in the urine, 665; artificial nutrition, 739
v. Stejskal, R., 240
Sternberg, S., 526
Stepanek, J. O., 502
Stern, H., 331
Stern, Heinrich, 496
Stern, L., 20
Stern, R., bile 329; ethereal sulphuric acids, 589
Steudel, H., mucin, 67; arginine, 97; glucosamine, 121; nucleic acids, 151; pyrimidine, bases, 152, 164–166
Stewart, C. W., 359
Stewart, G. N., blood, 236; muscle, 449, 453
Steyrer, A., muscle, 453, 472; urine, 546
Sticker, G., saliva, 343, 346
Stiles, P., 298
Stintzing, R., 467
Stockmann, R., 471
Stoffregen, A., 643
Stohmann, F., digestion of cellulose, 397; calorific determinations, 724
Stoklasa, J., fermentations, 21, 396, 523; lecithin, 146; glycolysis, 302; lactic-acid formation, 461
Stokvis, B. J., bile-pigments, 322, 323, 603, 653; benzoic acid, 588; urobilin, 604, 644; hæmatoporphyrin, 649
Stolnikow, J., 646
Stolte, K., 550
Stoltz, Fr., 278
Stone, W. E., 111
Stookey, L. B., gelatine, 79; glycogen, 292
Stoop, F., cystine, 93; serine, 93, 96
Storch, V., milk, 517, 539
Stradomsky, N., oxalic acid, 583, 630
Strashesko, N. D., 350
Strassburg, G., gases of the lymph, 251; carbon-dioxide tension, 709, 712
Strassburger, J., 410
Straub, W., glycosuria, 299; metabolism, 734, 754
Straus, J., 262
Strauss, Edw., spongin, 81, 82
Strauss, H., levulose, 118, 119, 184, 259; blood, 123; transudates, 259, 262; bile, 326; lactic-acid fermentation, 371; urine, 546
Strecker, A., lecithin, 143; bile-acids, 315
Strickler, E., 528
Strohmer, F., 445
Stusiewicz, B., 747
Struve, H., 649
Strzyzowski, C., 213
Subbotin, V., blood, 244; alcohol, 754
Sugg, E., 523
Suida, W., 334
Sulima, A. Th., digestion, 370, 400
O'Sullivan, C., 13
Sundberg, C., 356
Sundvik, E., glucosamine, 121; xanthine bodies, 157; uric acid, 568; conjugated glucuronic acids, 610, 632; chitin, 686; psylla alcohol, 692
Suter, F., protein sulphor, 28; thiolactic acid, 28, 74, 94
Suto, K., 663
Suzuki, W., 92
Svenson, N., 753
Swain, R. E., 103
Symmers, D., 614
Syniewski, V., starch, 126; dextrin, 128
Székely, S., 137
v. Szontagh, F., milk enzymes, 523; caseins, 529, 531
Szydlowski, Z., 361
Szymonowicz, L., 278

Takamine, J., 278
Tallquist, T. W., 749
Tammann, G., enzymes, 12, 14
Tangl, Fr., blood-serum, 189, 190; blood-analyses, 191, 236; blood-sugar, 297; 297; development of the egg, 511, 512, C : N = quotient, 628, 720
Tanret, Ch., 524
v. Tappeiner, H., cellulose, 397, 404; bile-acids, 426
v. Tarchanoff, J., bile pigments, 331; tata-proteid, 506
Tarulli, L., 544
Taveau, R., 278
Tawara, 764
Taylor, A. E., fatty liver, 283; fat formation, 443; lack of mineral bodies, 735
Tebb, Chr., reticulin, 81; glycogen, 289; amylolysis, 345, 388; intestinal invertase, 379; cholesterin, 487
Teeple, J., bilirubin, 320, 321
Teichmann, L., hæmin crystals, 211, 649
Tengström, B. St., bile, 311, 314
Terrat, P., 616
v. Terray, P., bile and putrefaction, 406, 407; oxalic acid, 582; lactic acid in the urine, 608
Terroine, E. F., 388
Terry, O. P., 342
Teruuchi, Y. , 551
Tetzner, E., saliva, 343

Thelen, M., 563
Thesen, J., isocreatinine, 455; indican, 593
Thiele, O., 613
Thiemich, M., 283
Thierfelder, H., galactose, 120, 484; barium, 166; digestion and microorganisms, 405; protagon, 482; cerebron and cerebrosides, 484, 485; sphingosin, 485; mammary gland, 514, 539
Thiroloix, J., 301
Thiry, L., intestinal fistula and intestinal juice, 377, 378
Thörner, W., 515
Thompson, W. H., 550
Thoms, H., 556
Thudichum, L. W., lecithins, 143; bile-pigments, 321; brain phosphatides, 480–482, 485; cerebrosides, 483–486; luteins, 505; urotheobromin, 580; urinary pigments, 601; alcohol in the animal body, 754
Tiedemann, F., 347
Tiemann, H., milk, 522, 528
Tigerstedt, K., 620
Tigerstedt, R., respiration apparatus, 713, 722; metabolism, 729, 730, 756, 758, 768
Tissot, J., 468
Tobler, L., digestion, 370; phosphaturia, 621
Toepfer, G., 617
Tollens, B., carbohydrates, 105, 111, 113, 117, 118; glucuronic acid, 123; urea, 556
Tolmatscheff, milk, 526, 531, 534
Tompson, F. W., 13
Toppelius, M., 563
Torup, S., carbohæmoglobin, 206, 207; globulins, 701
Tower, 81
Toyonaga, M., 286
Traube, M., oxidations, 3, 6, 8
Traube, W., 157
Treupel, G., urinary carbohydrates, 609, 659
Trifanowski, D., 326
Trillat, A., 19
Tritschler, F., 630
Troller, J., 353
Trommer, C., sugar test, 116, 655
Trümpy, D., 693
Tschenloff, B., 744
Tscherwinsky, N., 445
Tschirjew, S., 248
Tsuboi, J., 244
Tuczek, F., 346
Tüllner, H., 568
Turby, H., 378

v. Udránsky, L., diamines, 24, 615, 676; bile-acids, 312, 652; urinary pigments and humin substances, 601; sugar test, 659; cystine, 676
Uffelmann, J., 374
Uhlenhuth, 186
Uhlik, M., blood-crystals, 201, 203
Ulpiani, C., lecithins, 146, 147; brain, 480; uric acid, 568
Ulrich, Chr., 125
Ultzmann, R., 680
Ulzer, F., 137
Umber, F., proteoses, 55; nucleoproteids, 150, 381; transudates, 258; gastric juice, 352, 353; levulose, 665
Umikoff, N., 532
Underhill, F. P., 234
Urbain, V., 697
Ure, A., 635
Ussow, 393
Ustjanzew, W., 427

Vahlen, E., bile-acids, 316, 317
Valenciennes, A., muscles of cephalopods, 478; yolk-spherules, 509
Valenti, A., 532
de Vamossy, Z., 280
Vandegrift, G. W., 429
Vandevelde, A. J. J., 523
Vanlair, C., stercobilin, 320, 409
Vaubel, W., halogen protein, 30; Millon's reagent, 42; estimation of acetone, 673
Vauquelin, L. N., 583
Vay, Fr., liver proteid, 282; glycogen, 468
v. d. Velde, A., 588
v. d. Velden, R., 588
Velichi, J., 477
Vella, L., 377
Veraguth, O., 744
Verhaegen, A., gastric secretion, 353, 365
Verneuil, 349
Vernois, M., 534
Vernon, H. M., erepsin, 379, 380, 391; pancreas enzymes, 386, 388, 390, 391, 396; tryptic digestion, 394
Verploegh, H., creatinine, 563, 567
Verworm, M., 4
Viault, P., 245
Vierordt, K., spectrophotometry, 218; expiration air, 707
Vignò, L., 413
Vignon, L., 82
Vila, A., blood-pigments, 202, 205; musculamine, 457
Ville, J., blood-catalases, 20; blood-pigments, 202, 205, 212; fat absorption, 425; estimation of chlorine in urine, 616
Villiers, A., 615
Villiger, V., 6
Vincent, Sw., 478
Vines, S. H., plant-enzymes, 380, 390
Viola, E., 191
Virchow, R., amyloid, 69; hæmatoidin 216; suprarenal capsule, 330
Vitali, A., 649
Vitali, D., 616
Vitek, E., 21
Völtz, W., milk-corpuscles, 517; nutritive value of asparagin, 747
Vogel, J., pentoses, 110, 666; amylysis, 125, 344, 389; lactase, 379
Vogel, R., 467

Vogelius, 288
Voges. O., 548
Vohl, H., 458
Voirin, G., 597
Voit, C., glycogen formation, 291, 293, 420; bile and putrefaction, 406; fæces, 408; absorption, 413, 419, 424; fat formation, 442–445, 742; work and metabolism, 471–473, 759; nitrogen in meat, 444, 476, 720; urea formation, 553; elimination of phosphoric acid, 620; detection of milk sugar, 665; standard figures for dietaries, 728, 750, 764, 765, 767; nitrogen deficit, 718; oxygen requirements, 723; water content of the body, 734; starvation metabolism, 728, 729, 732, 738; mineral metabolism, 735; protein catabolism, 740–744, 751; organized and circulating protein, 742–744; nutritive value of gelatine, 745; proteoses, 746; asparagin, 747; water and metabolism, 753; salts, 753; dietaries, 764, 770, 771
Voit, E., fat determination, 137; glycogen, 293; bones, 438, 439; fat formation, 444, 445; heat of combustion, 727; starvation metabolism, 730, 732, 738; vegetable diets, 750; law of surface, 757
Voit, Fr., fermentation of sugar, 120; sugar elimination, 293, 420; thyroidea, 277; glycogen formation, 293; formation of fæces, 409; curare poisoning, 467; acetone bodies, 669, 670
Volhard, F., estimation of pepsin, 357; lipase, 363; estimation of trypsin, 392
Volhard, J., titration methods, 290, 581, 617
Volkmann, A. W., 735
van Voornveld, J. A., 246
Vossius, A., 331
Voswinckel, H., 690
Vozárik, A., acidity of urine, 544, 545
Vulpian, A., suprarenal capsule, 277

Wachsmann, M., 388
Wachsmuth, L., 260
Wälchli, G., 76
de Waele, H., 523
Wagner, B., 664
Wagner, H., extractives of the mucle, 455, 456
Wahlgren, V., biliary mucus, 310, 329; glycocholeic acid, 312
Wait, Ch., 471
Wakeman, A. J., 284
Waldvogel, R., uric acid, 571; acetone bodies, 669
Walter, Fr., urea formation, 551; blood-gases, 701
Walter, G., ichthulin, 71, 504
Walther, A., 382
v. Walther, P., fat absorption, 421, 423
Wanach, R., 220
Wang, E., 594
Wanklyn, J. A., 518
Wanner, Fr., 714
Warren, J., 469
Wasbutzki, M., 407
Wassermann, A., 186
Wassiliew, W., 387
Wawrinsky, R., 368
Waymouth Reid E., 419
Weber, 509
Weber, O., 439
Wedenski, N., carbohydrate of the urine, 608
Weidel, H., xanthin reaction, 160; carnine 456
Weigert, Fr., 98
Weigert, R., 185
Weinland, E., glycogen, 288, 293; antienzymes, 355, 372, 380; lactase, 379, 386; lactose in the intestine, 420
Weintraud, W., diabetes, 300; elimination of nitrogen, 554; phosphorus metabolism, 619
Weisbach, 486
Weisgerber, G., 710
Weiske, H., cellulose digestion, 397; bones, 438, 439; nutritive value of asparagin, 747
Weiss, J., alkalinity of the blood, 223; formation of sugar, 297; uric acid, 586
Weiss, H. R., trypsin, 392, 393
Weiss, Sigm., glycogen, 291, 293, 468
Weissberg, J., 6
Wells, H. G., 101
v. Wendt, G., metabolism, 719, 738
Wenz, J., 51
Werenskiöld, F., 529
Werigo, B., proteins, 38; respiration, 710
Wertheimer, E., bile, 329; pancreatic juice, 384
Werther, M., saliva, 347; glycogen, 467; lactic acid, 469
Westphal, C., 217
Wetzel, G., conchiolin, 81; histidine, 99
Weydemann, H., 32
Weyl, Th., protein crystals, 38; fibroin, 82; alanine, 84; carbon monoxide methæmoglobin, 207; cholesterin, 334; muscle work, 470; amniotic fluid, 512; creatinine, 565; benzoic acid, 588; nitrates, 623
Wheeler, H. L., iodogorgonic acid, 82; nucleic acids, 156; cytosine, 165
White, B., allantoin, 574, 584
Wichmann, A., proteins, 33, 181, 522
Widdicombe, J. H., 379
Wiechowski, W., hippuric acid, 586, 587
Wiener, H., autolysis, 23; uric acid, 18, 569, 571, 572, 573, 586; oxalic acid, 583
Wild, W., 7
Willdenow, C., 98
Williams, D., 397
Willstätter, R., 144
Wiman, A., 47
Windaus, A., histidine, 99; methylimidazole, 108; cholesterin, 334
Winkler, 441

Winteler, L., 309
Winter, J., estimation of hydrochloric acid, 376; milk, 530
Winterberg, H., ammonia, 241, 544; liver, 280; carbamic-acid intoxication, 552
Winternitz, Hugo, estimation of blood pigments, 217; quantity of hæmoglobin, 243; contents of the gall-bladder, 329; putrefaction, 405, 588; iodized fat, 442, 537, 538
Winternitz, M. C., purine-base enzymes, 158, 273, 571
Winterstein, E., amino-acids, 84, 87, 90; hexone bases, 97, 98, 100; inosite, 458; colostrum, 528; tunicin, 686; lecithans, 144
Wislicenus, J., 473
Wittmaack, K., 532
Wöhler, Fr., synthesis of hippuric acid, 635; of urea, 547; decomposition of uric acid, 573; allantoin, 583
Wörner, E., protagon, 482; cerebron, 485; creatinine, 563; uric acid, 578
Wohl, A., 106
Wohlgemuth, J., liver proteid, 96, 282; oxyaminosuberic acid, 96; oxydiaminosebacic acid, 101; pentoses, 109, 111, 112, 290; glycogen formation, 290, 292; sulphurized products of metabolism, 331; enzymes of the egg-yolk, 503; amino-acids, demolition of, 636
Wolff, E., 471
Wolff, H., glucosamine, 121; transudates, 262; melanins, 689
Wolff, L. K., 46
Wolffberg, S., glycogen formation, 293; respiration, 708, 709
Wolkow, M., 597
Woll, F. W., 516
Woltering, H., iron preparations, 245; liver-proteid, 282
Woods, H. S., 487
Wooldridge, L. C., tissue-fibrinogen, 141; stroma of blood-corpuscles, 194; coagulation of the blood, 228, 233, 234
Worm-Müller, J. ,blood, 244, 245, 248; sugar test, 655; estimation of sugar, 660, 662, 663
Wright, A., fibrin ferment and coagulation, 175, 226, 233; alkalinity of the blood, 223; phlorhizin diabetes, 298
Wroblewsky, A., fermentation, 10; pseudonuclein, 47; starch, 126; pepsin, 354, 358; gastric digestion, 370, 371; action of enzymes, 397; milk, 531
Wulff, C., purine bases, 161, 164, 580
Wurm, W. A., 690
Wurster, C., 625
Wurtz, A., lymph, 251; colloid, 499

Young, P. A., 325
Young, R. A., dextrin, 129; glycogen, 289
Yvon, P., 611
Zadik, H., 719
Zängerle, M., 500
Zahor, H., 646
Zaitschek, A., enzymes of milk, 523; casein, 521, 529, 531
Zak, E., 258
Zaleski, J., plant- and blood-pigments, 197, 214; blood-pigments, 211, 213, 214, 216; hæmopyrrol, 214; ammonia in the blood, 241, 551; in the glands, 624, 774; estimation of, 625; formation of urea, 553, 554; estimation of urea, 561; liver and acid formation, 554, 572
Zaleski, St., iron of liver, 282, 286; iron in sucklings, 535; reaction of the intestine, 407; milk, 536
Zalesky, N., bones, 436; samandarin, 692
Zalocostas, P., 81
Zander, E., 686
Zanetti, C. U., glucoproteid, 180; bile 310; ovomucoid, 508
Zangemeister, W., estimation of blood-pigments, 217; amniotic fluid, 513
Zaudy, 569
Zdarek, E., 688
v. Zebrowski, E., 342
Zeehuizen, H., 631
Zeidlitz, P. V., 656
Zeitler, X., 470
Zeller, A., elimination of chlorine, 616; destruction of chlorine compounds, 631
v. Zeynek, R., fat of dermoid cysts, 138, 502, 691; blood-pigments, 204, 205, 209; liver, 286; bile, 327; sarcomelanin, 689
Zickgraf, G., oxidation of proteins, 32, 78
Ziegler, 583
Ziegler, E., 635
Zillesen, H., 469
de Zilva, L., 387
Zimnitzki, S., 405
Zink, J., fats, 135, 437, 442
Zinoffsky, O., 197
Zinsser, A., 363
Zobel, S., 459
Zoja, L., oxyproteic acids, 31; elastin, 75, 76; ovalbumin, 507; urobilin, 604; uroerythyrin, 607; hæmatoporphyrin, 649, 650
Zsigmondy, R., 41
Zuelzer, G., lecithin, 147; myelin, 486; cutaneous respiration, 695
v. Zumbusch, L., 324
Zunz, E., digestion products, 53, 55; digestion, 370, 418; trypsinogen, 386; absorption, 413; extractives of meat, 457
Zuntz, L., 223
Zuntz, N., blood, 224, 243, 246; glycogen, 288; blood-sugar, 296, 469; phlorhizin diabetes, 298; digestion, 393, 427; protein assimilation, 412; muscle-fat,

463, 472; muscle metabolism, 467, 471, 473; high altitudes, 246, 694, 761; cutaneous respiration, 695; blood-gases, 697–699; alveolar air, 707: respiration, 710, 712, 713, 722, 723, 733; metabolism, 723, 729, 755, 757, 758, 761; nutritive value of proteoses, 746; of alcohol, 754; digestibility of bread, 762
Zweifel, P., ptyalin, 344; pancreatic diastase. 388; eclampsia, 608
Zwerger, R., 58, 59

4 –